PB84-162874

Removal of 'Giardia lamblia' Cysts by Drinking Water Treatment Plants

Washington Univ., Seattle

Prepared for

Municipal Environmental Research Lab.
Cincinnati, OH

Mar 84

U.S. Department of Commerce
National Technical Information Service
NTIS

PB84-162874

EPA-600/2-84-069
March 1984

REMOVAL OF GIARDIA LAMBLIA CYSTS BY DRINKING WATER TREATMENT PLANTS

by

Foppe B. DeWalle
Jogeir Engeset
William Lawrence
University of Washington
Seattle, Washington 98195

Grant No. R806127

Project Officer

Gary Logsdon
Drinking Water Research Division
Municipal Environmental Research Laboratory
Cincinnati, Ohio 45268

MUNICIPAL ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/2-84-069	2.	3. RECIPIENT'S ACCESSION NO. PB84 162874
4. TITLE AND SUBTITLE Removal of Giardia lamblia Cysts by Drinking Water Treatment Plants		5. REPORT DATE March 1984 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Foppe B. DeWalle, Jogeir Engeset, William Lawrence		8. PERFORMING ORGANIZATION REPORT NO
9. PERFORMING ORGANIZATION NAME AND ADDRESS Dept. of Environmental Health University of Washington Seattle, Washington 98195		10. PROGRAM ELEMENT NO. BNC1A 11. CONTRACT/GRANT NO. R806127
12. SPONSORING AGENCY NAME AND ADDRESS Municipal Environmental Research Laboratory - Cin., OH Office of Research and Development U.S. Environmental Protection Agency Cincinnati, Ohio 45268		13. TYPE OF REPORT AND PERIOD COVERED Final 9/78 - 3/82 14. SPONSORING AGENCY CODE EPA/600/14

15. SUPPLEMENTARY NOTES

Project Officer: Gary S. Logsdon (513) 684-7345

16. ABSTRACT

A study was conducted to evaluate the removal of Giardia lamblia cysts and cyst-sized particles by coagulation/sedimentation and filtration, or direct filtration using 2.3 L/min (0.6 gpm) pilot plants and by diatomaceous earth (DE) filtration using a 3.8 L/min DE pilot filter. The units were located at the University of Washington. The results were verified through field testing using a 75 L/min (20 gpm) pilot unit.

The study noted greater than 99.9% removal of spiked cysts under optimum conditions. Both the pilot unit and the field unit established the importance of a minimum alum dosage (10 mg/L), an optimum pH range, and intermediate flow rates of 4.9 m/hr (2 gpm/ft^2) to 9.8 m/hr (4 gpm/ft^2). Effluent turbidity and cyst-sized particles passing the filter increased rapidly when the above conditions were not attained or when sudden changes occurred in plant operation. When no coagulants were used during filtration, only 48% of the spiked cysts were removed, and 47% of the turbidity. A cyst spike in the pilot unit in Hoquiam using alum as coagulant resulted in an 81% cyst removal, and the spike at Leavenworth using a polymeric flocculant gave a 72.1% removal. DE filtration proved effective both for turbidity, particle, and cyst removal. The addition of 0.0075 mg/L nonionic polymer showed some improvement in efficiency. Cyst removals ranged from 99% to 99.99%.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 123
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

DISCLAIMER

The information in this document has been funded wholly or in part by the United States Environmental Protection Agency under assistance agreement number R806127 to the University of Washington. It has been subject to the Agency's administrative review, and it has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

FOREWARD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimonies to the deterioration of our natural environment. The complexity of that environment and the interplay of its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution, and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems to prevent, treat, and manage wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, to preserve and treat public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research and is a most vital communications link between the researcher and the user community.

This report presents the results and conclusions from pilot plant filtration research on the removal of Giardia lamblia cysts and cyst-sized particles from drinking water. Granular media filters and a diatomaceous earth filter were evaluated in this study.

Francis T. Mayo
Director
Municipal Environmental Research
Laboratory

ABSTRACT

A study was conducted to evaluate the removal of Giardia lamblia cysts and cyst-sized particles from Cascade Mountain waters. Methods included coagulation/sedimentation and filtration, or direct filtration using three 2.3 L/min (0.6 gpm) pilot treatment units and diatomaceous earth (DE) filtration using a 3.8 L/min (1 gpm/ft^2) DE pilot filter. The units were located at the University of Washington. The results were verified through field testing using a 75 L/min (20 gpm) pilot unit (Waterboy, Neptune Microfloc) in field trials at Hoquiam and Leavenworth, Washington.

The study noted greater than 99.9% removal of spiked cysts under optimum conditions, although removal percentages decreased greatly at lower spiking levels. Both the University of Washington pilot unit and the field unit established the importance of a minimum alum dosage (10 mg/L), an optimum pH range, and intermediate flow rates of 4.9 m/hr (2 gpm/ft^2) to 9.8 m/hr (4 gpm/ft^2). Effluent turbidity and cyst-sized particles passing the filter increased rapidly when the above conditions were not attained or when sudden changes occurred in plant operation. When no coagulants were used during filtration, only 48% of the spiked cysts were removed, and 47% of the turbidity. A cyst spike in the pilot unit in Hoquiam using alum as coagulant resulted in an 81% cyst removal, and the spike at Leavenworth using a polymeric flocculant gave a 72.1% removal. Producing a low turbidity filter effluent with alum or polymeric flocculant was difficult when the water temperature was 3° C. Further research in low temperature direct filtration is necessary to improve the removal efficiency under these conditions. DE filtration proved effective both for turbidity, particle and cyst removal. The addition of 0.0075 mg/L nonionic polymer showed some improvement in efficiency. Cyst removals ranged from about 99% to 99.99%.

This report was submitted in fulfillment of Grant No. R-8061/27 by the University of Washington under the sponsorship of the US Environmental Protection Agency. This report covers the period from September 1978 to March 1982, and work was completed at that date.

CONTENTS

Foreward iii
Abstract iv
Figures vi
Tables ix

1. Introduction 1
    Characteristics of Organism 1
    Characteristics of Disease 2
    Prevalence of Organism 4
    Giardiasis Outbreaks 5
2. Conclusions 11
3. Experimental Procedures 13
    Collection and Enumeration of Giardia Cysts 13
    Design and Testing of the 2.3 L/min. (0.6 gpm) Water Treatment Pilot Plants 22
    Testing of Coagulation/Filtration and Direct Filtration at the University of Washington 24
    Testing of Diatomaceous Earth Filter at the University of Washington 25
    Testing of Direct Filtration in Hoquiam and Leavenworth . . . 28
    Hoquiam Water Treatment Plant 29
    Leavenworth Water Treatment Plant 31
4. Results 32
    Method Evaluation: Collection, Enumeration of Giardia Cysts and QA/QC 32
    Testing of University of Washington Pilot Plant 41
    Testing of Coagulation/Filtration and Direct Filtration at University of Washington 47
    Testing of Diatomaceous Earth Filter at the University of Washington 73
    Testing of Direct Filtration in Hoquiam and Leavenworth . . . 78

References 100

Appendix 102

FIGURES

Numbers Page

1 Sucrose gradient technique to recover cysts from stool specimens 14

2 Modified sucrose gradient technique to recover cysts 15

3 Schematic of 293mm Millipore Filter Unit used to recover G. lamblia cysts from water 18

4 Procedure for recovery of G. lamblia cysts with the 293mm Millipore filter 19

5 Procedure for recovery of cysts from dilute water suspensions . . 20

6 Water treatment pilot plant at University of Washington 23

7 Schematic of the DE filter system 26

8 Cross section of coagulation, flocculation and mixed media filtration compartments of the Waterboy-27 30

9 Size distribution of serially diluted Giardia suspension in distilled water at (1) 5%, (2) 2.5%, (3) 1.25% and (4) 0.625% of the stock solution 33

10 Linearity of two counting methods for enumerating Giardia cysts . 34

11 Coefficient of variation for two methods used for enumerating Giardia cysts 35

12 Results of 47mm diameter membrane filter recovery test using Lake Union water spiked with Giardia cysts. (1) before recovery, (2) recovered cysts and (3) background counts 37

13 Percent recovery of Giardia cyst by different 47mm diameter membrane filters from two types of water 38

14 Percent recovery of cysts by 293mm diameter, 5µm pore size membrane filters, (A) Millipore and (B) Nuclepore 39

15 Effects of pH on the zeta potential of fixed G. lamblia cysts, (A) different suspensions and (B) same suspension 43

Numbers Page

16 Tracer evaluation of the rapid mix tanks 44

17 Tracer evaluation of the flocculation tanks 45

18 Tracer evaluation of the sedimentation tanks 46

19 Turbidity in filter influent and effluent of Run no. 4 52

20 Turbidity in filter influent and effluent of Run no. 5 53

21 Turbidity in filter influent and effluent of Run no. 6 54

22 Turbidity in filter influent and effluent of Run no. 7 56

23 Effect of alum dosage on direct filtration process, Filter B . . 58

24 Effect of alum dosage on direct filtration process, Filter C . . 59

25 Effect of pH on direct filtration performance, Filter B 60

26 Effect of pH on direct filtration performance, Filter C 61

27 Effect of pH increase on filter performance 63

28 Effect of flowrate on direct filtration efficiency, Filter B . . 64

29 Effect of flowrate on direct filtration efficiency, Filter C . . 65

30 Particle removal at different filter depths 66

31 Sampling schedule for 20L filter effluent sample at different filtration rates 68

32 Percentage of total number of filter effluent cysts present in a 20L sample collected according to Figure 31 69

33 Effect of alum dosage on cyst removal 72

34 Effect of pH on cyst removal 74

35 Characteristics of DE filter run with Celite 503 filter aid at 20 mg/L body feed 75

36 Typical data from a DE filter run using Hyflo Super-Cel as filter aid. Body feed rate, 20 mg/L 76

37 Typical data from a DE filter run using Celite 512 as filter aid. Body feed rate, 20 mg/L 77

Number Page

38 Effect of alum dosage on particle and turbidity removal during field work at Hoquiam 80

39 Effect of pH on particle and turbidity removal during field work at Hoquiam 82

40 Effect of pH changes during Run no. 9 at Hoquiam 83

41 Effect of filtration rate on particle and turbidity at Hoquiam . 85

42 Effect of high filtration rate on filter performance at Hoquiam. Alum dosage 15 mg/L, pH 6.7 and filter loading 15 m/hr (6.1 gpm/ft^2) 86

43 Relationship between effluent turbidity and particle removal at Hoquiam 87

44 Relationship between effluent turbidity and median particle removal 88

45 Turbidity removal at Hoquiam Water Treatment Plant 90

46 Effect of alum dosage and pH on turbidity removal at Hoquiam Water Treatment Plant 91

47 Effect of alum dosage on particle and turbidity removal at different temperatures during field work at Leavenworth 93

48 Effect of pH on particle and turbidity removal at different temperatures during field work at Leavenworth 94

49 Effect of Cat Floc T polymer dosage on particle and turbidity removal and rate of headloss buildup at Leavenworth 96

50 Frequency distribution of particle removal at different effluent turbidities during alum and polymer treatment at Leavenworth . 97

51 Effect of polymer dosage on turbidity removal at Leavenworth Water Treatment Plant 98

TABLES

Number		Page
1	Summary of Laboratory Recovery Rates of *G. lamblia* Cysts with Millipore Pellicon Cassette Unit	40
2	Zeta Potential (Electrophoretic Mobility) of Buffered Formalin Fixed *Giardia lamblia* Cysts at Varying pH Values and Cyst Concentrations	42
3	Zeta Potential of a Fixed *Giardia lamblia* Cyst Suspension at Different pH Values	42
4	Results of a Single Dose Spike of *Giardia* Cysts into Flocculation Compartment of Pilot Plant - Run #1	48
5	Results of Continuous Spike of *Giardia* Cysts into Pilot Plant - Run #2	50
6	Results of Continuous Spike of *Giardia* Cysts Directly Introduced into Dual Media Filters - Run #3	51
7	Performance of Each Filter Run with Cysts Added Directly to the Filter	57
8	Cyst Removal During Direct Filtration at UW Pilot Plant	71
9	Filter Runs with Cysts Using DE Filter	79

SECTION 1

INTRODUCTION

A study was undertaken to evaluate the removal of Giardia lamblia cysts by drinking water plants. The first phase of the study was devoted to a laboratory-scale evaluation of Giardia removal efficiency by coagulation, flocculation, and filtration. In addition, a diatomaceous earth filter was tested. The second phase consisted of a pilot-scale evaluation of Giardia cyst and cyst-size particle removal from drinking water at locations in the State of Washington that were suspected of harboring cysts in the raw water.

All laboratory water treatment plant experiments were conducted with unfiltered Seattle tap water to which cysts were added. The cysts that were used to spike the water were isolated from the feces of human giardiasis patients. The cysts were recovered from the spiked water using membrane filtration techniques. Giardia cysts present in the membrane retentate were enumerated with a hemacytometer and a Coulter Counter.

Currently Giardia lamblia is the most commonly identified pathogen in waterborne outbreaks in the U.S. and the protozoan is especially predominant in the Pacific Northwest, Rocky Mountain states and New England.

CHARACTERISTICS OF ORGANISM

Giardia lamblia is a pathogenic intestinal parasite found in humans and certain animals. The multiflagellated protozoa belong to phylum Sarmastigophora, subphylum Mastigophora, class Zoomastigophorasida, order Diplomonadorida, family Hexamitidae, and subfamily Octomitinae. The organism was first observed by Antony van Leeuwenhoek in 1681 while studying his own feces (Dobell, 1932). During the mid and latter part of the 19th century, the organism was observed and studied by many workers. The genus was named by Joseph Kunstler in 1882, but until Charles Wardell Stiles established the name Giardia lamblia in a letter to Kofoid and Christiansen (Kofoid and Christiansen, 1915), the organism had been synonymously known as Giardia intestinalis, Giardia duodenalis, or Giardia enterica.

The organism has two stages in its life cycle: the reproductive trophozoite stage and the dormant cyst stage. The trophozoite is pear-shaped with a broad anterior end that comes to a blunt point posteriorly. The dorsal surface is convex, whereas the ventral surface, which contains a large sucking disk, is somewhat concave. Another name for the sucking disk is the striated disk, because of the striated appearance of the pellicle which is caused by its alternating light and dense lines. The trophozoite is 9 to 21 um long, 5 to 15 um wide, and 2 to 4 um thick. The organism is bilaterally symmetrical with eight flagella. Its basal bodies arise near the midline at the level of the two anterior vesicular nuclei. Two of the flagella emerge anterolaterally, two posterolaterally, two ventrally, and two caudally. The parasite has no true axostyle, as has been previously reported. Rather, what has been observed is the intracytoplasmic axonemes of the ventral flagella and the associated groups of microtubules. Two media bodies are composed of bundles of microtubules arranged either irregularly or sometimes united in ribbons. Their function is obscure, though it has been suggested that they may help support the posterior end of the organism, be involved in its energy metabolism, or have something to do with formation of the new sucking disk. The trophozoites reproduce by binary fission (Levine, 1979). The ovoid- to ellipsoidal-shaped cyst of G. lamblia is surrounded by a hyaline cyst wall approximately 0.3 um thick and composed of thin fibrous elements interspersed with fine particles (Sheffield and Bjorvatn, 1977). The cyst is smaller than the trophozoite (8 to 12 x 7 to 10 um). A peripherally situated lacunar system is separated from the plasma membrane and cyst wall by a thin layer of cytoplasm. The flagellae of the trophozoite are believed lost or reabsorbed upon encystment. But the intracytoplasmic portions (axonemes) of at least six flagellae are retained. Newly formed cysts have two nuclei, whereas mature cysts have four. Although nuclei have been observed in close apposition, none have been seen dividing. Exactly when division or doubling of the other organelles takes place is uncertain. But during excystation, two trophozoites emerge from each cyst.

CHARACTERISTICS OF DISEASE

G. lamblia has been the most common pathogenic intestinal parasite in the United States ever since the Centers for Disease Control (1979) initiated the Intestinal Parasite Surveillance Report in January 1976. An estimated 7 percent of the adult population harbor the parasite (Schultz, 1975). The intestinal disease caused by G. lamblia is called giardiasis. Symptoms of the disease appear from 2 to 35 days after exposure to the cyst. In most cases, however, the incubation period is about 1 to 2 weeks. The cyst is the only form of the organism's two life stages infectious to man. If ingested, the vegetative trophozoite will be destroyed during passage through the early stages of the digestive system, whereas the cyst will survive until it reaches the small intestine. The environmental conditions there support the emergence of the trophozoites, which divide rapidly and can build up to enormous numbers. A single diarrheic stool can contain 14 billion parasites, and a stool from a moderate infection may contain 300 million cysts (Chandler and Read, 1961).

In most Giardia infections, the diagnosis can be made by stool examination. In some cases, it may take more than one specimen to confirm the disease when using direct smear and concentration techniques. When it becomes necessary to examine more than one stool specimen, the probability for a positive identification will increase by examining stools on alternate rather than consecutive days. Diagnosis also appears to be easier in early acute infections rather than established ones. In the acute stage, stools are frequently watery or loose and may contain mostly trophozoites and few cysts because of rapid bowel transit.

In a series of controlled experiments with prison volunteers, Rendtorff (1954) studied different epidemiological problems of various human intestinal protozoans, among them G. lamblia. One of the objectives of the study was to establish the minimum number of cysts capable of producing an infection, by feeding known numbers of cysts to the volunteers. Of the five men who received only 1 cyst, no one became infected. When the dosage was increased to 10 cysts per person, both volunteers became positive, thus indicating that the critical number for infection is somewhere between 1 and 10 cysts per person.

The acute stage of infection is manifested by a sudden onset of explosive, watery, often foul smelling diarrhea, marked abdominal flatulence and distention, foul gas, nausea, anorexia, and cramps, which are usually upper or midepigastric. Less frequently there is vomiting, chills, low-grade fever, headache, and belching. The acute stage usually lasts only 3 to 4 days and is often not recognized at the time as being due to giardiasis. In some cases, the acute stage may last for months, leading to malabsorption, debility, and significant weight loss. This latter situation appears to be more common in children than adults which perhaps explains why giardiasis was formerly considered a disease of childhood.

Acute infections can develop into long-standing subacute or chronic infections. The most common symptoms include intermittent mushy and foul smelling stools, abdominal flatulence and distention, primary upper intestinal cramps, nausea, anorexia, foul belching, heartburn, headache, constipation, weight loss, and fatigue. The symptoms may either be persistent or recurrent and are usually milder than during the acute stage of the infection. Although most individuals with giardiasis are symptomatic, many are asymptomatic and may never become symptomatic. But the potential exists in some for development of intermittent chronic symptoms.

The protozoan does not lyse or rupture host cells, but appears to feed on mucous secretions. A dense coating of trophozoites on the intestinal epithelium interferes with the absorption of fats and other nutrients, which can trigger the onset of disease. The gallbladder may become infected, which can cause jaundice and colic. A few cases of urticaria have been reported (Webster, 1958; Wolfe, 1979), and erythema multiforme (Konenenko, 1976) and arthritic symptoms (Goodbar, 1977) have been found associated with giardiasis.

Usually the parasite disappears spontaneously from the infected individual but, that may take from a few days to several months. Once a person has recovered from giardiasis, there are indications that some resistance to re-infection has developed. The degree of resistance may vary among individuals, and there is some uncertainty as to whether it is of permanent or temporary nature.

Most of those infected with G. lamblia today are treated with drugs. The most effective and commonly prescribed are quinacrine (Atabrine) and metronidazol (Flagyl), but both have potential problems. Quinacrine may cause serious toxic effects in a small percentage of those taking it, including toxic psychosis, vomiting, fever, and exfoliative dermatitis. Metronidazol is a suspected carcinogen and mutagen. Neither of the drugs has been proven safe for use by pregnant women. If used at all during pregnancy, they should be administered only to those women with severe symptoms definitely attributable to giardiasis where benefit is judged to outweigh potential risk.

PREVALENCE OF ORGANISM

Although the incidence of giardiasis does vary from one area to another, G. lamblia is a cosmopolitan parasite. According to the public health laboratories in the United States, the states with the largest percentage of G. lamblia positive stool specimens in 1978 (Centers for Disease Control, 1979) were Arizona, Arkansas, California, and Washington. Of the total number of stool specimens examined in these four states, more than 8 % were positive for G. lamblia. These figures do not necessarily mean, however, that the same states top the list of waterborne outbreaks or total number of reported cases.

Waterborne outbreaks of giardiasis have occurred primarily in the mountainous areas of this country particularly in New England, the Pacific Northwest, and the Rocky Mountains. Colorado has experienced more outbreaks than any other state and this probably reflects increased surveillance and investigation. Another possible explanation for the higher incidence of giardiasis in the mountainous areas is the general concept about water quality. High mountain lakes and streams are assumed to be free from pollution and, therefore, when used as domestic water supplies, chlorination is usually the only treatment. Often the chlorine dosage is low and adequate contact time is not always provided. The potential for G. lamblia to be present in the mountain regions, is increased by the heavy recreational usage in many of these areas. When considering the high percentage of asymptomatic carriers in the adult population, there is a possibility of direct human contamination of the water or indirect contamination through cross-transmission to animals. In the lowland areas the water source is known to be contaminated and appropriate treatment facilities are built, establishing the barrier necessary to protect the public.

Many isolated cases of giardiasis involve people who use the outdoors for recreation or work and drink untreated water. Many conceive crystal clear lakes and sparkling streams in the mountains with no permanent human habitation as the ultimate in water purity. Little thought is given to the often great potential for contamination of these waters by fellow users of the area or by wildlife.

A study aimed at identifying animal reservoirs of G. lamblia in Colorado and New Mexico (Davis and Hibler, 1979) found a significant number of beaver, coyotes, cattle, cats and dogs infected with Giardia. When exposed to G. lamblia cysts of human origin, the majority of the beaver, bighorn sheep, dogs, pronghorn deer, mule deer, and raccoons became infected. Human volunteers and dogs ingesting cysts from a naturally infected beaver and mule deer were shedding cysts within one to two weeks after exposure, thus emphasizing the potential for cross-species transmission of Giardia.

Another study, to assess the prevalence of Giardia infection in aquatic mammals in Washington State (Frost et al., 1980), found a significant number of positive beaver and muskrat. During the three year investigation, the percentage of Giardia-positive animals increased each year, reaching 19.0 percent for the beaver and 42.6 percent for the muskrat. The juvenile beaver and muskrat showed a higher positivity than the adults and judging by the number of cysts excreted, the beaver had a higher level of infection than the muskrat. Positive animals were found both in protected and nonprotected watersheds, suggesting that pathogen-free surface waters may be difficult to find.

The information on cyst survivability in water is limited. Working with human volunteers, Rendtorff and Holt (1954) found the cysts to retain their infectivity after 16 days of storage at 8°C. Davis and Hibler (1979) successfully infected dogs with cysts that had been stored in the refrigerator for 21 days. Some of the earliest work on infectivity and storage was done by Fantham and Porter (1916). A female kitten was fed food contaminated with G. lamblia cysts from a stool specimen that had been kept for 74 days. No information was given on how the stool specimen was stored. After nine days, cysts were recovered from the cat feces and the animal showed signs of diarrhea. Boeck (1921) found Giardia to be viable after 32 days when stored in distilled water at 12 to 20°C, and at least 66 days when sealed under a cover slip on slides. The eosin-stain technique used by early researchers including Boeck to determine viability, however, is of questionable value.

GIARDIASIS OUTBREAKS

During the period 1971 to 1978 a total of 24 outbreaks of waterborne giardiasis were reported, affecting more than 7,000 persons. Although reporting has generally improved in recent years, more waterborne outbreaks occur than are reported. The majority of these outbreaks were caused by the drinking of untreated surface water or surface water in which chlorination was the only treatment. Only a few involved filtered water.

One of the first outbreaks of gastroenteritis in which G. lamblia was implicated as the probable etiological agent, occurred in Portland, Oregon, from October 1954 to March 1955 (Veazie et al, 1979). The Oregon State Board of Health estimated that at least 50,000 cases occurred during that period. Much controversy concerning the pathogenicity of G. lamblia existed at that time. In an effort to pinpont the cause of the illness a survey was made of a group of people, most of whom were symptomatic. The bacteriological studies revealed no enteric pathogens and the incidence of intestinal protozoa other than G. lamblia did not differ from what had been found in similar groups in the past. However, there was an abnormally high prevalence of Giardia infection. The flagellate was found in 44% of those studied during the outbreak, in contrast to 7% of those examined during nonepidemic periods. The source and mode of spread were never satisfactorily determined, but the water supply could well have been involved. Heavy rains with a subsequent increase in water turbidity was reported during the period of the outbreak.

The first waterborne outbreak of giardiasis documented in the U.S. occurred at Aspen, Colorado, during December 1965 through January 1966 (Moore et al, 1969). A survey of 1,094 skiers who had vacationed in Aspen during the two months showed that at least 123 had developed symptoms characteristic of giardiasis. The city received approximately half of its water from a distant mountain creek and half from three wells. Both sources were chlorinated, but coliform contamination had been noticed intermittently during the winter. A survey of the sparsely populated creek area revealed no obvious possibility of sewage contamination. However, tracers placed in the Aspen sewerage system were detected in two of the three wells. An engineering evaluation discovered leaking sewer mains near the wells and G. lamblia cysts were isolated from the sewage in these lines. A parasitologic survey of Aspen residents detected only a modest level of Giardia infection.

The largest outbreak of giardiasis and the first where a G. lamblia cyst was recovered from the municipal water supply, occurred in Rome, New York, during November 1974 to June 1975 (Shaw et al, 1977). It was also the first time that water from an outbreak had been shown to infect laboratory animals. A total of 350 residents had laboratory-confirmed giardiasis and an epidemiologic study estimated that more than 5,300 persons may have been symptomatic.

The first sign of an epidemic surfaced in early January 1975 when G. lamblia was identified in stool specimens from eight of 23 persons in Rome with gastroenteritis. Since early November, however, local health department personnel had been investigating an increased incidence of diarrhea. During this investigation, G. lamblia was the only pathogen commonly identified. A random household survey in the city indicated an overall attack rate of 10.6%. No correlation was found between illness and daily activity, animal contact, or consumption of food, but a significant association was discovered between having giardiasis and using water from the city system as opposed to using water from private wells.

Rome used a surface water source located several miles to the north of the city. From the intake at Fish Creek the water was piped to a reservoir where chlorine and ammonia were added, first at the inlet and then at the outlet as the water entered the distribution system. No other treatment was provided. The water at most sampling points in the distribution system was negative for coliforms but the total bacteria count was high, indicating an inadequate disinfection. In an attempt to isolate G. lamblia from the municipal water supply, raw water was filtered through a small pressure filter. At the end of each filter run the sand filter was backwashed. The backwash water was collected, coagulated and flocculated and the floc allowed to settle. The sediment was used for microscopic examination or aliquots were fed to pathogen-free dogs. In one of the sediment samples examined microscopically one G. lamblia cyst was found. Further evidence of contamination of the raw water supply by the parasite was obtained when G. lamblia was found in some of the dogs.

The source of infection was never established. However, the watershed was found to be more heavily populated than city officials expected and there were some questions about the sanitary disposal procedures at some of the settlements. No animal survey was conducted to assess the potential for contamination by wildlife.

Until 1976, all the reported outbreaks of giardiasis in which municipal water supplies were implicated, had involved surface water with chlorination as the only treatment. In late April and early May of 1976, local physicians in Camas, Washington, reported the occurrence of approximately 25 cases of giardiasis. This became the first reported outbreak involving a filtered water supply (Kirner et al., 1978). The epidemiological investigation that followed, showed that approximately 600 people had clinical signs of the infection.

The city of Camas used both surface water and deep well water as sources of supply. The surface water sources were Boulder Creek and Jones Creek which came from adjoining watersheds. Both sources were generally of excellent quality including low turbidity based on existing standards. From the intake, the water flowed by gravity to a direct filtration system. Unlike most direct filtration plants, the injection of pretreatment chemicals occurred immediately prior to the two multimedia pressure filters. Chlorine was added in a transmission main about 1.5 hours upstream of the water treatment plant. Chlorine was not added to the filtered water except during three separate failures of the upstream chlorination equipment. The seven wells were primarily used to augment the surface water supply during periods of high demand or when the flow in Boulder and Jones Creeks was low. As a safety measure the well water was chlorinated, but no additional treatment was necessary.

Most of the confirmed giardiasis cases initially reported were located in areas of the community most likely to receive surface water. Hence, the surface water system was suspected of being the source of the G. lamblia cysts. A survey of the watersheds indicated no human habitation and most of the roads were found to be in very poor condition making access difficult. No obvious source of contamination was observed but signs of beaver activity were in evidence. With the help of professional trappers a total of seven beavers were trapped in the watersheds, three of which were found to be infected with G lamblia.

Of the treated water samples collected for bacteriological examination during the outbreak, only one was unsatisfactory. However, G. lamblia cysts were recovered from both the raw and treated water at different locations and times. An inspection of the water treatment plant in search of clues that might explain how cysts could escape into the distribution system revealed a cross connection between raw and filtered water in the coagulant feed line. Further, there had been loss of media in both filters and the coarse garnet had regions of mounding which could cause short circuiting. The effectiveness of the coagulation process was questioned because of insufficient control of the coagulant feed rate and the short detention time prior to filtration. A subsequent analysis of the filtration process using a particle counter indicated a 75% removal of particles in a 7 to 16 micron size range which incorporates the size of a G. lamblia cyst.

On three different occasions during the month of April, the chlorination equipment on the raw water main had been out of service due to mechanical difficulties. During that time the chlorination was performed manually, but after review of the emergency chlorination, procedures, it was concluded that large amounts of water arrived at the treatment plant without adequate chlorination. The time differential between the chlorination equipment failures and the majority of detected giardiasis cases correlated closely to the incubation period for the disease. Even so, the chlorination equipment failures cannot explain all the cases since the earliest signs of the outbreak were evidenced prior to the first breakdown at the chlorination plant.

The second outbreak of giardiasis to involve a filtered surface water supply occurred in Berlin, New Hampshire, in the spring of 1977. In a two week period in early April, 100 cases of G. lamblia infection were diagnosed. By the time the outbreak subsided in the middle of May, estimates based on subsamples of persons in community-wide surveys indicated that 3,450 people had experienced gastronintestinal illness, 1,656 of which were symptomatic for giardiasis (Lopez et al., 1980). Among the remaining segment of the population exhibiting no signs of gastrointeritis, an estimated 5,197 people had asymptomatic G. lamblia infection. The first 100 confirmed cases of giardiasis were randomly distributed in the city. Since a preliminary analysis revealed no events or meals common to these cases, a waterborne epidemic was suspected.

Berlin had two independent sources of water, the Upper Ammonoosuc River and the Androscoggin river. The watersheds of the two rivers had no known large point sources or discharges. Hunting, fishing and other forms of recreation were permitted but no public sanitary facilities were available on the upper Ammonoosuc watershed. Water from the two sources was treated separately and supplied to identifiable areas within the distribution system, although some areas received a mixture. The older of the treatment plants, receiving water from the Upper Ammonoosuc River, consisted of eight pressure filters. No provision was made for chemical pretreatment, and turbidity monitoring equipment was not available. The water was chlorinated prior to distribution. The Androscoggin plant was put in service just before the outbreak, replacing an older filtration plant. The new plant provided conventional treatment including chemical coagulation, clarification, rapid sand filtration, and chlorination.

G. lamblia cysts were first identified in the Berlin water system by the Androscoggin Valley Hospital Laboratory (Lippy, 1978). Water drawn from a laboratory tap was passed through an improvized gauze filter overnight and the filter material was found positive for cysts when examined microscopically. Samples of raw and finished water at both treatment plants and of water collected from the distribution system were also G. lamblia positive. A survey conducted in the Ammanoosuc watershed to determine the source of contamination disclosed a beaver lodge upstream of the treatment plant intake. Four beavers were eventually trapped, but only one had G. lamblia infection. Since there was ample opportunity for human fecal contamination of the raw water, it could not be determined whether this animal was an unlucky victim of water contaminated with G. lamblia of human origin or whether the beaver served as a major contributing source of the organism in the water. A similar survey of the Androscoggin watershed was not seriously considered because of its large size and thus the source of the G. lamblia cysts at the Androscoggin treatment plant was never determined. However, because of the recreational activities in the area the human aspect could not be completely ruled out. Furthermore, residential sewage disposal violations were known to occur along the upstream portion of the river.

The operation at both plants was studied to develop remedial action that would prevent G. lamblia cysts from passing through the treatment process. At the Ammonoosuc plant, no chemicals were used to condition the water prior to filtration which made cyst passage through the filters very likely. Mudballs and mounding of the filter medium in some of the filters further impaired the efficiency of the filtration process. The chlorine dosage and contact time were inadequate to inactivate the G. lamblia cysts.

The Androscoggin plant had experienced some floc carry-over to the filters, but this was not considered a serious problem, It was discovered, however, that air bubbles were escaping from the joints in the slab of the backwash channels during air scour of the filters. The escape of air through the joints indicated the possibility for raw water to seep through the joints during filtration and to contaminate the filtered water. The possibility was confirmed by a static hydraulic test of the backwash channel. It showed that over 3% of the plant output was not filtered.

Another outbreak of giardiasis involving filtered surface water occurred at Leavenworth, Washington, from January through May 1980. A survey conducted in early May indicated that as many as 600 people might have been affected. Among the city's water customers 27% in the group surveyed had experienced diarrhea with symptoms characteristic of giardiasis (Austin and Harter, 1980). For people on private wells the incidence was only 3%, and each of the persons with Giardia infection had been exposed to Leavenworth water either through restaurants or work.

A source of supply for Leavenworth is the Icicle River. Raw water turbidity is normally less than 0.5 NTU. There was no permanent human habitation above the water intake, but the watershed was open for recreation with several Forest Service campgrounds located on the river. However, during the time period of the outbreak these camps were not likely to have been inhabited and the sewage disposal for the camps was contained and not likely to contaminate the river. From the intake structure the water flowed by gravity to a direct filtration plant. The plant was designed for chemical addition, coagulation, and filtration, but because of the low raw water turbidity no chemical pretreatment had ever been practiced.

Surveillance and filtering activities were conducted at the water treatment plant. G. lamblia cysts were recovered from the filtered water. This implicated the water supply as responsible for the outbreak. The actual source of the contamination was never determined. According to personnel at the Forest Service ranger station, there were many good beaver habitats at higher elevations, but signs of beaver activity in the area had not been reported. The inspection at the treatment plant also revealed a significant loss of filter media which required that all four filters be rebuilt.

SECTION 2

CONCLUSIONS

The study demonstrated that to achieve cyst removal efficiencies greater than 95%, unit process operation must be optimized. Process variables of primary importance were coagulant type, coagulant dosage, and the pH of the raw water when alum was used as the primary coagulant. Raw water turbidity, filtration rate, and sudden changes in plant throughput were shown to be of secondary importance. Raw water temperature was a key variable when temperatures were <5° C. Cold water temperatures slowed the rate of alum floc formation which resulted in a significant amount of floc forming throughout the depth of the filter and effluent piping.

During field operation with the USEPA mobile pilot plant, the effect of low water temperatures on the coagulation and flocculation process was particularly noticeable when using alum, but also with polymers. It was felt that if the detention time in the flocculator had been longer, higher removal efficiencies may have been possible.

The polymers tested as primary coagulants did not perform as well as alum. The removal efficiency was generally 10% less for turbidity and 11% less for cyst-sized particles. On the other hand, the filter runs were longer and the necessity for close pH monitoring experienced during alum treatment was not required.

The role of the operator is critical in order to optimize unit process operation. Sudden changes in the raw water may require immediate adjustments of chemical feed and pH as was demonstrated during field operation. Under such conditions, good plant records become important.

Jar tests were initially used to obtain information on optimum plant operating conditions. However, because the pilot plants were operated as direct filtration plants, the data from the jar tests were found to be of limited value since the tests provided no information about the filterability of the floc. Rather than relying on the jar test, optimum operating conditions could be determined quickly and reliably by stepwise changing the major process variables one at a time while monitoring the filtered water quality. This approach was also used successfully to provide important information to the full scale plant during conditions of rapidly changing raw water quality.

Diatomaceous earth (DE) filtration is an effective method for removing G. lamblia cysts. With a precoat of 1.0 kg/m^2, more than 99.35% of the cysts added were removed at the beginning of the filter run. As the thickness of the filter cake increased the removal ranged from 99.61 to 99.96%. The effieiency of the DE filter can be improved by the addition of a nonionic polymer, added with the body feed. A 0.0075 mg/L dosage of Magnifloc 985N increased the cyst removal from 99.94 to 99.99%. Larger dosages would reduce the length of the filter run.

Information obtained from pilot plant work such as this can be a valuable aid in improving a full-scale plant operation. Equally important, it can be a tool to gather information useful in the design of water treatment facilities.

SECTION 3

EXPERIMENTAL PROCEDURES

COLLECTION AND ENUMERATION OF GIARDIA CYSTS

Experimental procedures only are described in this section. The results of these experimental procedures may be found in Section 3, Results.

Collecting Giardia Cysts from Human Feces from Giardiasis Patients

The fecal samples were received from the State of Washington Parasitology Laboratory, Department of Social and Health Services. The cysts were separated from the human feces using a sucrose gradient technique as modified from the one used by Sheffield and Bjorvath (1977). These feces had been examined and confirmed for presence of *Giardia* cysts by Ms. Yvonne Fichteneau. The stools had been preserved by the addition of 5% formalin to inactivate pathogenic bacteria.

To isolate the cysts, the feces were emulsified in approximately 20 mL distilled water and were passed through 3 layers of gauze (60 to 100 um mesh equivalent). The procedure is outlined in Figure 1. The filtrate was subsequently centrifuged at 1400 rpm for 3 min. The supernatant was poured off and the sediment was resuspended in 5 mL distilled water. This suspension was pipetted onto a discontinuous density gradient of 5 mL each of 1.5 M, 1.0 M, 0.75 M and 0.50 M sucrose in a conical centrifuge tube followed by centrifugation at 2200 rpm for 30 min. The cysts were then collected from the H_2O - 0.5 M sucrose and 0.5 M-0.75 M sucrose interface, by means of a capillary pipette. In our procedure, microscopic examination of the cysts showed the absence of any extraneous debris and further filtration through 20 um and 5 um filters was not necessary. The cysts in this sucrose solution were then diluted with distilled water to 1 L and were kept at 4° C. The technique was gradually modified as listed in Figure 2.

Cyst Recovery Using the 47 mm Filter Technique

Aliquots of 10 mL and 20 mL of distilled, tap and Lake Union water samples were spiked with known concentrations of *Giardia* cysts as determined by hemacytometer. The aliquots were subsequently filtered through 47 mm diameter 5 um pore size membrane filters to isolate the cysts.

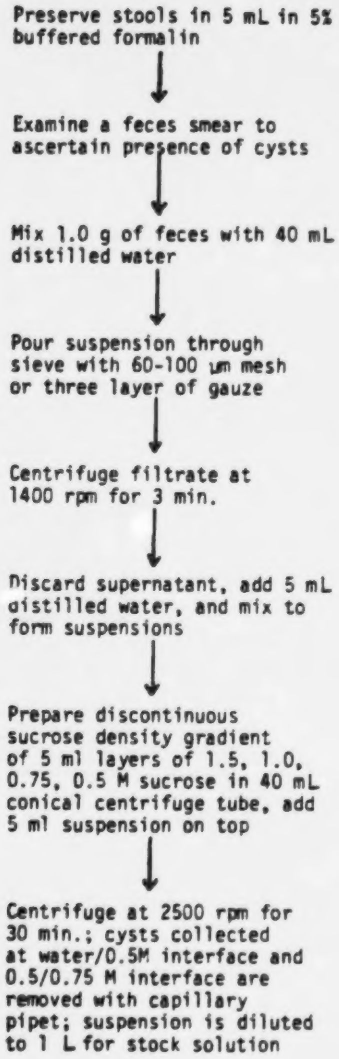

Preserve stools in 5 mL in 5% buffered formalin
Examine a feces smear to ascertain presence of cysts
Mix 1.0 g of feces with 40 mL distilled water
Pour suspension through sieve with 60-100 µm mesh or three layer of gauze
Centrifuge filtrate at 1400 rpm for 3 min.
Discard supernatant, add 5 mL distilled water, and mix to form suspensions
Prepare discontinuous sucrose density gradient of 5 ml layers of 1.5, 1.0, 0.75, 0.5 M sucrose in 40 mL conical centrifuge tube, add 5 ml suspension on top
Centrifuge at 2500 rpm for 30 min.; cysts collected at water/0.5M interface and 0.5/0.75 M interface are removed with capillary pipet; suspension is diluted to 1 L for stock solution

Figure 1. Sucrose gradient technique to recover cysts from stool specimens.

Examine a feces smear to ascertain presence of cysts.

↓

Transfer feces to 2000 mL beaker. Add distilled water to 500 mL and homogenize.

↓

Pour suspension through 3 or 4 layers of cheesecloth. Transfer liquid to 250 mL round centrifuge bottles and centrifuge at 650 xg (2100 rpm) for 2 min.

↓

Aspirate off supernatant and resuspend sediments with distilled water. Pour into four 50 mL centrifuge tubes, make volume to 45 ml in each tube.

↓

Wash by centrifuging at 650 xg (2100 rpm) for 2 min. Discard supernatant and resuspend sediments. Repeat using 2 drops Dawn dishwashing liquid per 15 mL of suspension.

↓

Wash with distilled water until supernatant is reasonable clear.

↓

Resuspend sediments in 25 mL distilled water and layer onto 25 mL, 1.0 M sucrose, in two 50 mL centrifuge tubes. Centrifuge at 800 xg (2400 rpm) for 10 min.

↓

Aspirate off 3/4 of supernatant appearing above the band of cysts. Pour remainder of suspension into 50 mL centrifuge tube and centrifuge at 800 xg (2400 rpm) for 2 min.

↓

Aspirate off supernatant and resuspend cysts at the bottom and repeat washing procedure twice.

↓

Final sediments are resuspended and stored at 4°C.

Figure 2. Modified sucrose gradient technique to recover cysts.

Elution of the cysts from each membrane filter was accomplished by placing the filter with cysts in a small flask, adding equivalent amounts of distilled water and electrolyte totaling initial volume of water that was filtered. The flask was then gently agitated so that a flow of water passed over the membrane's upper surface.

The effluent was examined for presence of *Giardia* by direct examination of effluent after centrifugation and by use of the Coulter Counter.

Methodology for Cyst Enumeration

Two counting techniques were used for the enumeration of the *Giardia lamblia* cysts. The first technique was microscopic counting using different counting chambers, and the second was an electric current displacement technique using a ZBI Coulter Counter and Channelyzer (Coulter Electronics, Hialeah, FL) calibrated to measure particle densities in the *Giardia* size range (8 to 12 um). The instrument measured the reduction in current between two sides of a small orifice before and during the time that a small particle passes through the opening. The current reduction was proportional to particle volume.

A Clay Adams Model 4011 Spencer Bright-Line Counting Chamber was used for the microscopic counting. Three alternative means for counting the cysts were used, depending upon their density in the solution. The cysts were counted in a volume of 0.02 mm^3 for suspected high densities or when *G. lamblia* cyst counts exceeded 20,000 cysts/mL. The multiplication factor is 50,000 times the number of cysts counted to give cysts per mL. The cysts were counted in a volume of 0.1 mm^3 for moderate densities in the range of 5,000 to 20,000 cysts/mL. The multiplication factor is 10,000 times the number of cysts counted to give cysts per mL. The cysts were counted in a volume of 0.9 mm^3 primarily for low densities. The multiplication factor is 1111 times the number of cysts counted to give cysts per mL; i.e., less than 5,000 cysts per mL. The specimens were stained with 5% Lugol's Iodine prior to pipetting into the hemacytometer. A low detection limit in a large amount of water was accomplished by concentrating the cysts and particles by passing the water through a membrane filter and resuspending cysts and particles in a small volume.

The electric current displacement method used a Coulter Electronics Coulter Counter particle counter, model ZBI with a 100 um aperture tube. A 0.9% Isoton solution was used both as diluting medium and electrolyte solution to allow flow of the electric current. In this method, the counts were based on the current interruption when a particle passed through the aperture, and were made per 0.5 mL of cyst-containing solution. A size frequency distribution Channelyzer coupled to the Counter was used to verify the counts of particles in the same size range as *Giardia*. This counting technique was not specific for *Giardia* cysts.

The cyst-sized particles in the water samples were enumerated with the Coulter Counter. Replicate counts were made, and means were calculated and compared to the mean values of the initial concentrations.

Cyst Recovery Using the 293 mm Membrane Filter Technique

Stock suspensions of 10^5 to 10^6 cysts/mL of Giardia lamblia cysts were prepared and stored at 4° C.

Appropriate volumes of these stock suspensions were then added to 10 L of distilled water to give different final concentrations. Nitrogen gas was used to pressurize the stainless steel vessel to pass the water through the filter unit (5 um pore size) at 10 psi (Figure 3). The time to filter 10 L through the membrane averaged 2.5 minutes.

The filter was then removed and carefully placed in a shallow plastic container of slightly larger diameter than the filter. Distilled water (0.5 L) was then added and the entire assembly agitated by means of a small shaker for 3 min (Figure 4).

A two-step concentrating method was selected to achieve a final volume of about 5 to 15 mL. This involved spinning of the 0.5 L wash in a centrifuge for 20 min at 1,000 rpm, about 20 mL of the centrate in each of four tubes. Respinning of the 80 mL in two conical tubes under the same conditions and collecting and combining the two 5 to 7 mL sediments permitted the sample to be enumerated by the Coulter technique. The technique was gradually modified to that listed in Figure 5.

Evaluation of Membrane Cassette Unit

The Millipore Pellicon Cassette Unit was a multiple surface area cassette filter. The unit contained nine 465 cm^2 (0.5 ft^2) Millipore membranes with a pore size of 1.2 um stacked on top of each other, separated by plates of acrylic plastic. The filtration rate was 0.07 m/hr (0.03 gpm/ft^2). The design of the unit was such that a retentate recirculated by a peristaltic pump with a retentate/filtrate ratio of 1:2 enabled a continuous flow of water over the filter area. Particles greater than 1.2 um in diameter remained in the retentate, the volume of which was readily controlled.

The Millipore Pellicon Cassette Unit was tested at the Ryderwood, Washington, reservoir in which beavers had been sighted and G. lamblia cysts had been detected in the upstream sediment. The unit was hooked up to the raw water intake inside the treatment plant with chlorination as the only preceding treatment. Over a three hour period, 93 L were passed through the unit with a retentate/filtrate ratio of 1:2. 100 mL of the retentate and wash were kept and examined for G. lamblia cysts by the following procedure. The concentration step represents a 98% volume reduction.

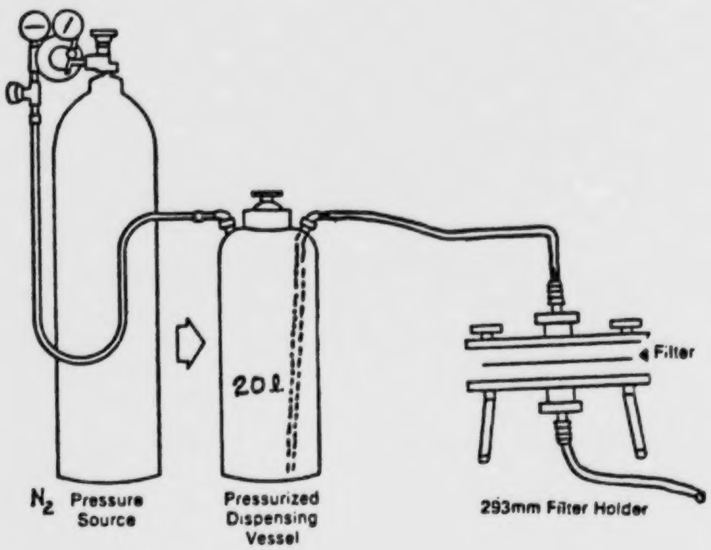

N2 Pressure Source
Pressurized Dispensing Vessel
20ℓ
Filter
293mm Filter Holder

Figure 3. Schematic of 293mm Millipore Filter Unit used to recover _G. lamblia_ cysts from water.

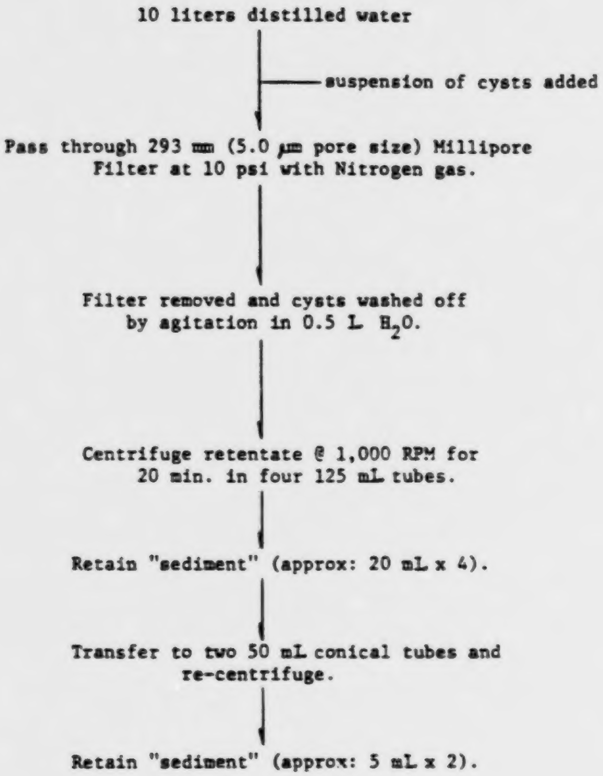

10 liters distilled water
suspension of cysts added
Pass through 293 mm (5.0 µm pore size) Millipore Filter at 10 psi with Nitrogen gas.
Filter removed and cysts washed off by agitation in 0.5 L H_2O.
Centrifuge retentate @ 1,000 RPM for 20 min. in four 125 mL tubes.
Retain "sediment" (approx: 20 mL x 4).
Transfer to two 50 mL conical tubes and re-centrifuge.
Retain "sediment" (approx: 5 mL x 2).

Figure 4. Procedure for recovery of _G. lamblia_ cysts with the 293mm Millipore filter.

Pass suspension through 293 mm (5.0 μm pore size) Nuclepore Filter at 10 psi with nitrogen gas. 0.2 μm filter on nitrogen tank.

↓

Remove filter and place in shallow dish. Cysts washed off by agitation of filter in 250 mℓ H_2O for 3 min. (platform shaker, Toothmaster Company, Racine, Wisconsin).

↓

Remove filter and rinse thoroughly.

↓

Centrifuge retentate at 350 xg (1500 RPM) for 10 min. in eight 50 mℓ conical bottom tubes.

↓

Aspirate off supernatant from each tube to 8 mℓ final volume. Transfer remaining volume to two 50 mℓ centrifuge tubes and recentrifuge

↓

Aspirate off supernatant from each tube to 5 mℓ final volume. Transfer remaining volume to one 15 mℓ centrifuge tube and centrifuge at 350 xg (1500 RPM) for 10 min.

↓

Aspirate off supernatant to 1 mℓ final volume.

Figure 5. Procedure for recovery of cysts from dilute water suspensions.

1. 50 mL of retentate centrifuged at 2000 rpm for 10 min.

2. Approximately 5 mL of sediment was passed through a discontinuous sucrose gradient (Sheffield and Bjorvatn, 1977).

3. The volumes of water between the H_2O - 0.5 M layer and 0.5 - 0.75 M layer were pipetted and examined under a microscope at 280 X for Giardia cysts.

4. The above procedure was also used for 50 mL of the wash from the filter unit.

Evaluation of Electrophoretic Mobility

Tests for electrophoretic mobility (EM) and zeta potential (ZP) were carried out to determine how it varied for formalin fixed Giardia cysts at different pH values using a Zeta Meter. The experiments were conducted using a plexiglass Riddick type II electrophoresis cell (Zeta Meter, Inc., New York, NY) with a 4.4 mm diameter cell tube and cell constant of 62. The cell had a platinum-irridium anode and cathode. Measurements were made at a distance of 0.147 diameters from the tube wall, which is the distance of no electro-osmotic fluid-flow. The voltage used for the experiments varied from 200 volts for a 0 to 300 micromho/cm to 50 volts for 700 to 1500 micromho/cm suspensions. Solutions of higher conductivity experienced more rapid thermal overturn due to heating of the solutions and the tube contents had to be replaced more often. A total of 10 or more individual cysts was measured in each batch with regard to their travel distance in the cell tube. As the distance between the electrodes was 10 cm, the voltage decline ranged from 20 to 5 volts/cm. The EM is calculated as

EM = Cyst Travel Distance/Time Interval divided by Volt/Electrode Distance

with the units of um/sec/volt/sec. The present study used a 98 um tracking distance for each cyst. The EM was converted to ZP (M volts) using the Helmholtz-Smoluchowski multiplication factor expressed as:

$$ZP = EM \cdot 4\pi \cdot V_t / D_t \cdot f(K_a)$$

(V_t = vixcosity of liquid; D_t = dielectric constant of liquid;

$f(K_a)$ = Henry relaxation correction)

The pH was measured with a Model 5 Corning pH meter (Corning, Glass, Corning, NY), standardized daily with pH buffer, while the turbidity was measured with a continuously recording low range Hach 1720A turbidimeter (Hach Chemical Co., Loveland, CO and standardized daily as suggested in the manual. To verify the readings of the flow-through turbidimeters, grab samples of the influent and effluent were analyzed daily on a DRT-100 (H.F. Instruments, Ft. Meyers, FL) bench top turbidimeter.

DESIGN AND TESTING OF THE 2.3 L/MIN (0.6 GPM) WATER TREATMENT PILOT PLANTS

The University of Washington pilot plant used chlorinated unfiltered Seattle tap water as "raw" water. In addition, it could be supplied by Lake Union water pumped from the lake adjacent to the campus. It was constructed from 3/4 in plywood, coated with fiberglass. The unit consisted of three individual but identical treatment plants, each designed for 2.3 L/min (0.6 gpm). This design allowed the plant operator to vary the capacity of the pilot plant and thereby the surface loading on the filters while keeping flow conditions and all the design factors identical at all times (Figure 6).

The pilot plant was 137 cm (54 in) wide, 229 cm (90 in) long, and 122 cm (48 in) high. Physical dimensions of the individual units and design factors are given below.

Rapid Mix

The dimensions were 15.2 cm by 15.2 cm (6 in) with a maximum water depth of 22.2 cm (8.75 in). At maximum depth the theoretical detention time was 2.3 min at 2.3 L/min flowrate A variable speed impeller produced G-values ranging from approximately 300 sec^{-1} to 1000 sec^{-1}.

Flocculators

The flocculator consisted of three compartments, each 21.6 cm (8.5 in) by 22.9 cm (9 in) and 35.6 cm (14 in) deep, measured from the overflow weir, producing a 23.2 min theoretical detention time. Calculated G-values ranging from 30 sec^{-1} to 150 sec^{-1} could be attained by changing the speed and surface area of the paddle blades making tapered flocculation possible.

Sedimentation Basins

The sedimentation basins were 45.7 cm (18 in) wide, 182.9 cm (72 in) long, and 91.4 cm (36 in) deep, measured from the overflow weir. With the 2.3 L/min (0.6 gpm) design flow rate, the theoretical detention time was 5.6 hr and the surface loading 3.9 m/d (96 gpd/ft^2). The tanks were designed with a baffled inlet zone and could be shortened to decrease detention time and increase surface loading by means of a divider wall.

Filter Columns

The study used two 10.8 cm (4.25 in) diameter plexiglass filter columns fitted with Turbitrol FC Media consisting of 50.8 cm of 0.92 mm effective size anthracite (UC=1.28) and 25.4 cm of 0.40 mm effective size sand (UC=1.30). The columns had headloss taps at 10.2 cm (4 in) intervals.

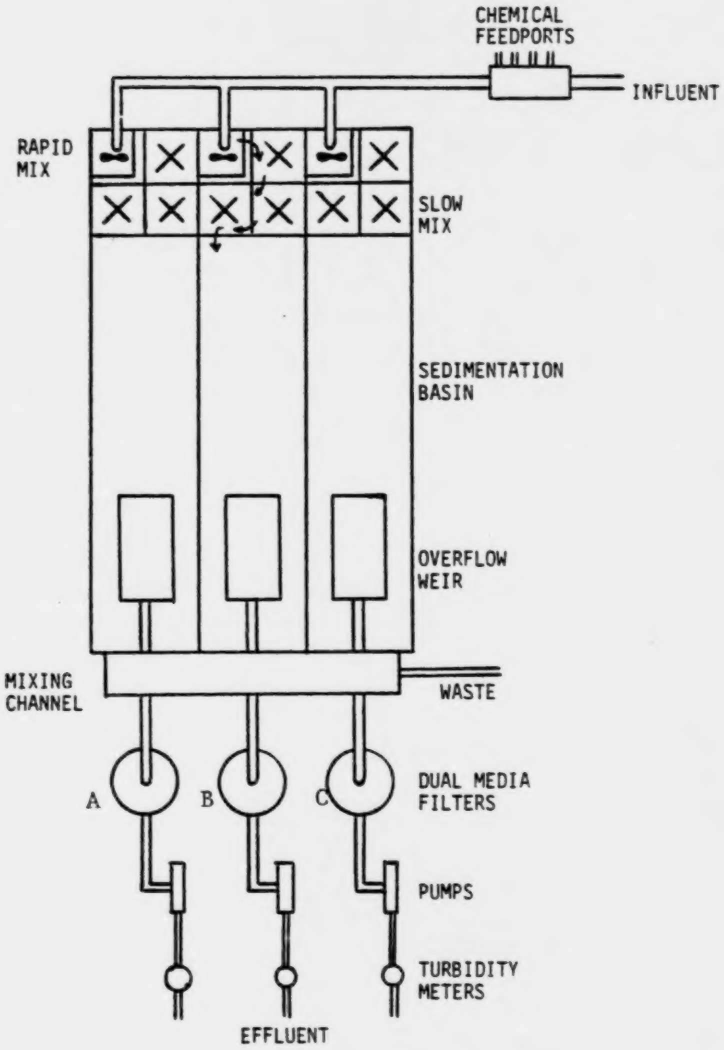

CHEMICAL FEEDPORTS
INFLUENT
RAPID MIX
SLOW MIX
SEDIMENTATION BASIN
OVERFLOW WEIR
MIXING CHANNEL
WASTE
A
B
C
DUAL MEDIA FILTERS
PUMPS
TURBIDITY METERS
EFFLUENT

Figure 6. Water treatment pilot plant at University of Washington.

Detention Time Testing

Conductivity tests were done to determine the retention times in the rapid mix and flocculation tanks. Sufficient amounts of NaCl were added to the tanks and conductivity was measured at fixed time intervals.

The retention time for the sedimentation tank was determined spectrophotometrically using an inorganic blue dye to prevent salt stratification. Samples were taken every 10 to 15 min and transmittance was measured by a Bausch and Lomb Spectronic 20 spectrophotometer.

It seemed reasonable to assume that the rapid mix tank and to some degree the flocculation tanks would behave as completely stirred reactors. The sedimentation tank on the other hand, would most likely show a combination of characteristics, some typical of a plug flow and others of a completely mixed reactor.

The tracer was added in the same manner for all retention time tests. A concentrated solution of tracer was metered into the line feeding water to the tank being tested. This was continued until the tracer concentration in the reactor had reached a constant level, at which time the feed was discontinued and the sampling of the reactor effluent initiated. Effluent samples were collected until practically all the tracer had been displaced from the reactor.

To determine the actual retention times of the various processes the concentration of tracer in the effluent was plotted vs. time on rectangular coordinates. The resulting tracer decay curve was divided into segments of equal time increments. The moment (time x concentration) of each segment about the origin was then computed and the sum of moments was divided by the sum of the concentrations to give the actual retention time.

Additional analyses were performed to obtain information on the flow regime and general performance of the tanks. If $F(t)$ is the fraction of tracer retained in the tank for a duration less than time t, then the fraction remaining in the tank longer than time t must be $1-F(t)$. For a single compartment, completely mixed reactor,

$$1-F(t)=e^{-(t/T)}$$

where T is the theoretical retention time. A semilog plot of $1-F(t)$ vs. t/T for a completely mixed reactor would yield a straight line. Deviations from a straight line, if any, could be used to provide information on the tank's dead space ratio and plug-flow and mixed-flow fractions by employing the relationship derived by Rebhun and Argaman (1965).

JAR TESTS

Jar tests using 3 L beakers and unfiltered Seattle tap water (chlorinated Tolt Reservoir water) were done to determine the optimum alum dosage during the water treatment pilot plant run. The test was done at 100 rpm rapid mix for 2 min, followed by slow mix at 30 rpm for 20 min. pH was adjusted with 0.1 N NaOH to 6.5. The jar test was done at two different sets of alum dosages with settling times of 30 min and 60 min. The turbidity of each sample was tested after settling.

Continuous testing with Giardia spiking

Continuous runs with the coagulation/filtration pilot plant used alum dosages of 10 mg/L with pH kept at 6.7 by addition of lime. The rapid mix was run at 500 rpm while the slow mix was kept at 22 rpm ($G=48\ sec^{-1}$). Figure 6 shows the cyst introduction and water sampling points.

The raw water flow rate was 2.3 L/min (0.6 gpm) with a corresponding filter loading rate of 4.9 m/hr (2 gpm/ft^2). Using unfiltered chlorinated Seattle tap water, initial particulate concentrations of the "raw" tap water and initial turbidity were also recorded prior to the start of the run.

Prior to spiking of the influent Seattle tap water with a *Giardia* cyst suspension, particulates/mL in the cyst-size range were established.

Spiking with *Giardia* was carried out at different points in order to establish removal efficiencies for each sequence of unit processes.

a) A single spike of cysts introduced into the first flocculation compartment of unit A.

b) A continuous dose of cysts introduced at the chemical feed port A_2, just ahead of entry into the rapid mix compartment.

c) A continuous dose of cysts bypassing flocculation-sedimentation and introduced into Filters B and C to determine losses in the filtration step alone.

TESTING OF DIATOMACEOUS EARTH FILTER AT THE UNIVERSITY OF WASHINGTON

Diatomaceous Earth (DE) Filter Performance

The DE test filter was a 0.1 m^2 (1 ft^2) pressure filter, operated at 3.8 L/min (1.0 gpm). A schematic of the filter system is shown in Figure 7. The operation of the filter consisted of three steps: precoating, filtration, and filter cleaning.

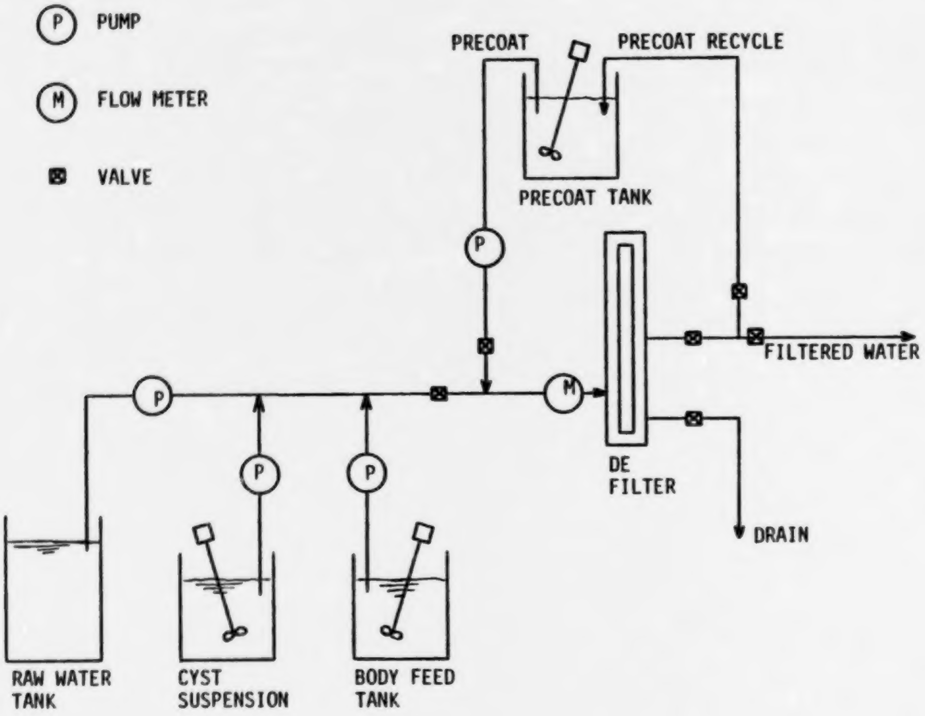

PUMP
FLOW METER
VALVE
PRECOAT
PRECOAT RECYCLE
PRECOAT TANK
FILTERED WATER
DE FILTER
DRAIN
RAW WATER TANK
CYST SUSPENSION
BODY FEED TANK
P
M

Figure 7. Schematic of the DE filter system.

In the precoat tank a slurry was prepared by adding the desired amount of diatomite to tap water. The slurry was recirculated through the filter at high rate while keeping the contents of the tank well mixed. A gradual buildup of diatomite on the filter septum could be observed and the water in the tank finally became clear and free of diatomite.

While positive pressure was maintained in the filtration chamber, the appropriate valves were opened and closed to change from precoat mode to filtration mode. During filtration, a small amount of diatomite body feed was continuously added to the raw water. This addition of fresh diatomite to the precoat filter cake meant that layers of clean diatomite were constantly rejuvenating the filter and thereby slowing down the headloss buildup due to particles plugging the filter cake pores. The thickness of the filter cake steadily increased during the run.

Filter runs were terminated when headloss exceeded 30 psi. The filter cake was removed from the septum and the spent diatomite discharged to waste. Septum and filtration chamber were carefully sluiced to make the filter ready for a new precoating.

The initial work with the DE filter was aimed at determining the amount of precoat required for adequate initial reduction of turbidity and particles in the 8 to 12 um range. Several different grades of diatomite, obtained from the Manville Products Corp., Denver, CO, were used. The amounts of precoat material applied to the septum ranged from 0.5 kg/m^2 (0.1 lb/ft^2) to 1.2 kg/m^2 (0.24 lb/ft^2). The results indicated that 1.0 kg/m^2 (0.2 lb/ft^2) would be adequate for all grades of diatomite, giving a 56 to 96% and 70 to 91% initial reduction in turbidity and cyst-sized particles, respectively. For the very fine grades such as Standard Super-Cel and Filter-Cel smaller quantities of precoat did not result in a significant increase in turbidity or cyst-sized particles in the filtered water. However, this finding was more of academic interest. Because of the relatively high initial headloss, the finest grades were not judged to be good candidates for full scale water treatment applications.

The initial runs with the DE filter were made without the addition of G. lamblia cysts to the raw water. The runs were designed to gain knowledge about the filter's performance with respect to particle and turbidity removal for different grades of diatomite. In addition, the amount of body feed was varied from 10 to 40 mg/L to investigate its effect on the rate of headloss buildup across the filter cake. Influent and effluent turbidities were monitored continuously with a Hach Model 1720A turbidimeter (Hach Company, Loveland, CO), while particle analyses were performed on influent and effluent grab samples using a Coulter Counter (Coulter Electronics, Inc., Hialeah, FL). Occasionally during some of the late runs in this series, difficulties were experienced in maintaining a constant flowrate through the filter. Most often the flow would increase over a period of time, thereby increasing the particle load on the filter and decrease the cake porosity since the body feed rate remained constant.

Diatomaceous Earth (DE) Filtration with Giardia lamblia cysts

The cysts used for the DE runs were extracted from stool specimens as described earlier. The concentration of the stock solution ranged from 1.0 x 10^5 to 4.6 x 10^5 cysts/mL, and was stored at 4° C until needed.

Cysts were added to the raw water at the same location as the body feed, either as a slug or as a constant continuous dosage. The slug contained a total of 3.0 x 10^6 cysts, added in 10 sec using a FMI Lab Pump (Fluid Metering, Inc., Oyster Bay, NY). For the continuous cyst addition, the parasite was metered into the raw water line with a Buchler Polystaltic Pump, Model 2-6100 (Buchler Instruments, Inc., Fort Lee, NJ). Different raw water cyst concentrations were used during these runs, ranging from 1.5 x 10^5 to 9.0 x 10^5 cysts/L.

The filter effluent sampling schedule was determined from a series of tests in which a salt solution was added to the raw water in place of cysts. The conductivity of the filter effluent was monitored continuously to determine: 1) how fast a 10 sec slug would pass through the filter, and 2) the time required to reach a constant effluent concentration when a continuous dosage was added to the filter influent. It was found that the entire slug would have reached the filter effluent in 10 min. That meant, in order to trap all the cysts escaping the filter, a 38 L sample would need to be collected. This was not an unreasonably large volume to process by the technique developed for this study. When a constant dosage was added to the raw water, the effluent concentration had attained its maximum and constant level after 10 min. By adding cysts for 15 min and sampling the filter effluent during the last 5 min, a 19 L sample containing an average effluent concentration of cysts was collected.

All cyst runs used Hyflo Super-Cel as filter aid. Based on results from preceding runs, without cyst addition, a 1.0 kg/m^2 (0.2 lb/ft^2) precoat and 20 mg/L body feed was judged most suitable for the 3.8 L/min filtration rate and raw water quality. During two of the four runs a 0.0075 mg/l dosage of the nonionic polymer Magnifloc 985N was added to the raw water for the duration of the run.

TESTING OF DIRECT FILTRATION IN HOQUIAM AND LEAVENWORTH

The last part of the study was used to validate the laboratory results in the field by using a mobile pilot plant. In addition, the pilot plant was to be compared with the full-scale plant to determine any discrepancies. All plants were intended to operate at conditions giving maximum cyst removals. The mobile pilot plant was tested at Hoquiam and Leavenworth, Washington, by treating a portion of the raw water. A comparison was also made between water quality generated by the pilot plant and the drinking water generated by the city water plant at each location.

The tests were conducted with a USEPA pilot drinking water treatment unit, the Waterboy-27 (Neptune Microfloc, Corvallis, Oregon) which was modified by extending the depth of the sand filter compartment by 83.8 cm (33 in) to provide for more headloss buildup and prevent negative pressures within the filter as shown in Figure 8. The upper boundary of the filter bed was dropped from 76.2 cm (30 in) to 124.5 cm (49 in) below the top of the unit. At Hoquiam the water was tapped from the water main through an unused chlorine injection port. Transportation of the raw water to the pilot plant by a 7.6 cm (3 in) line was provided by the pressure of the main. Water was pumped into the plant by two centrifugal pumps in series able to deliver a maximum of 75.7 L/min (20 gpm). After injection of chemicals the water was passed through three static in-line mixers, Model 2-50-541-5 (Kenics, Danver, MA), whereafter it entered the flocculator, which provided for an 8 min detention time at the common operating condition of 62 L/min plant flow (4.1 gpm/ft^2 in the filters). The water overflowed ito the filter compartment with a 78.7 cm (31 in) average water head above the filter. The filters consisted of 45.7 cm (18 in) MS-4 anthracite (e.s. 1.0 to 1.1 mm, u.c. < 1.7), 22.9 cm (9 in) of MS-6 sand (e.s. 0.42 to 0.55 mm, u.c. < 1.8), 7.6 cm (3 in) of MS-21 fine garnet (e.s. 0.18 to 0.28 mm, u.c. < 2.3), 7.6 cm (3 in) of MS-22 course garnet (e.s. 1 to 2 mm), 10.2 cm (4 in) of 0.95 cm (3/8 in) gravel, and 12.7 cm (5 in) of 1.9 cm (3/4 in) gravel (Neptune Microfloc, Corvallis, Oregon). The top support plate was perforated with 0.63 cm (0.25 in) openings 5.1 cm (2 in) apart from center to center to provide a total perforated area of 45.5 cm^2 (0.049 ft^2). Support gravel was located below the top plate followed by the bottom support plate perforated by 0.36 cm (0.14 in) openings 3.8 cm (1.5 in) apart from center to center, to provide a total perforated area of 47.4 cm^2 (0.051 ft^2). The filter effluent was then pumped to the 4731 L (1250 gal) backwash water tank which had an overflow at the top. At the end of the filter run the 246 L/min (65 gpm) backwash water pump delivered the 3690 L (975 gal) effective liquid volume to the bottom of the sand filters at a rate of 67.2 cm/min (16.5 gpm ft^2) for 15 min. The backwashing resulted in a 23% bed expansion which was less than the 50% expansion commonly used.

HOQUIAM WATER TREATMENT PLANT

Hoquiam is located in Grays Harbor County, Washington, approximately 21 kilometers (13 miles) from the Pacific Ocean and 80 kilometers (50 miles) north of the Columbia River and the Oregon border. The city has two main sources of water: Davis Creek and the west fork of the Hoquiam River. A third source, the Little Hoquiam River, is used only in case of emergency and bypasses the treatment plant.

The present water treatment plant at Hoquiam was completed in 1975. It was designed for a maximum flow of 11,400 m^3/day (3 mgd) and serves a population of approximately 10,500. It was a conventional plant providing coagulation, flocculation, sedimentation and filtration. At maximum flow the detention time in the flocculator was 6.5 min. From the flocculation basin the water overflowed into the rectangular sedimentation basin which provided 49 min retention at maximum flow. The clarified water was pumped to the three mixed media filters which were operated at a maximum filtration

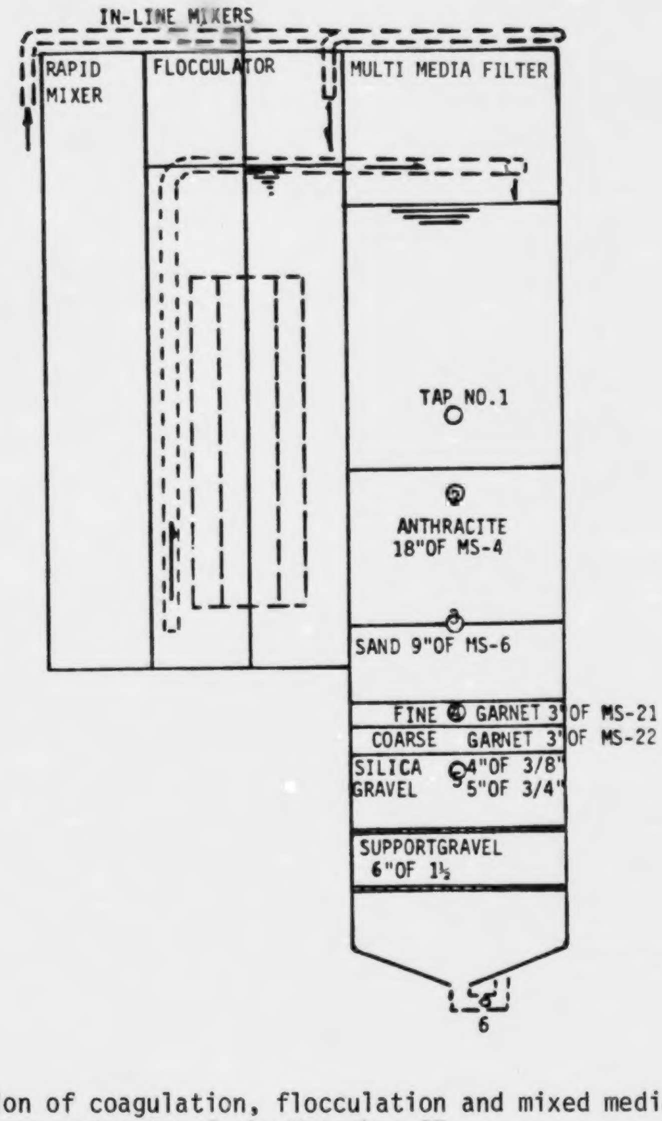

IN-LINE MIXERS
RAPID MIXER
FLOCCULATOR
MULTI MEDIA FILTER
TAP NO.1
ANTHRACITE 18"OF MS-4
SAND 9"OF MS-6
FINE GARNET 3"OF MS-21
COARSE GARNET 3"OF MS-22
SILICA GRAVEL 4"OF 3/8" 5"OF 3/4"
SUPPORTGRAVEL 6"OF 1½
6

Figure 8. Cross section of coagulation, flocculation and mixed media filtration compartments of the Waterboy-27.

rate of 12.2 m/hr (5 gpm/ft^2). Backwash was initiated by loss of head through the filters, when operating in automatic mode. Once a backwash cycle was initiated, it automatically backwashed each filter in sequence. Half of each filter was backwashed at a time. The backwash rate was 36.6 m/hr (15 gpm/ft^2) and the water was supplied by clearwell pumps. The filtered water was chlorinated in the clearwell and pumped to the city reservoir.

The plant normally used alum as the primary coagulant, sometimes in combination with a nonionic polymer as a coagulant aid. During periods of low turbidity, however, only polymer was used as a coagulant. The same polymer was also used as filter aid. Soda ash was used for pH control during coagulation and flocculation and for final pH adjustment in the clearwell.

LEAVENWORTH WATER TREATMENT PLANT

Leavenworth is located at the eastern foothills of the Cascade Mountain range near the Wenatchee River in Chelan County, Washington. The two sources of water used by the city are Icicle Creek and shallow wells, with Icicle Creek the main source for the city's 2,400 residents. Because of the dry climate, the per capita water usage is very high compared to the west side of the mountains.

The flow to the 13,300 m^3/day (3.5 mgd) direct filtration plant was controlled by an electric butterfly valve operated by signals from the storage reservoir just outside the city. The polymer used as coagulant was added directly to the 30.5 cm (12 in) raw water line before it entered the baffled flocculator. The retention time at maximum flow was about 9 min. From the flocculator the water flowed via the inlet flume to the four mixed media filters operated at a rate of 12.5 m/hr (5.1 gpm/ft^2).

Unlike many other plants, the filter operation was controlled by siphons. The siphoning was initiated by applying vacuum and the siphon was broken by allowing air to be sucked in, all of which was controlled by a series of solenoid valves. The filters were backwashed one at a time. As the headloss increased, the water level above the filter media would rise until it made contact with a sensor. At that time the inlet siphon would be broken and the backwash siphon initiated. The three filters remaining in the filter mode would supply the backwash water.

The filtered water was chlorinated in the clearwell and flowed by gravity to the storage reservoir. A booster pump was available for use if necessary, when the plant was operated at high flow rates.

SECTION 4

RESULTS

METHOD EVALUATION: COLLECTION, ENUMERATION OF GIARDIA CYSTS AND QA/QC

Statistical Evaluation of Cyst Enumeration Techniques

The linearity or proportionality of both counting methods was evaluated by using a 1:10 diluted stock suspension containing approximately 4×10^8 cysts/L as measured with a hemacytometer and Coulter Counter followed by a sequential 1:2 dilution to obtain 10%, 5%, 2.5%, 1.25%, 0.625% and 0.313% of the original stock solution. The dilution series were made in triplicate with distilled water. Each aliquot was counted five times and the counts for each sample were averaged and subtracted from the background count. A counting example is shown in Figure 9.

The results of the serial dilution are shown in Figure 10 for both counting methods, and indicate that some nonlinearity is observed for the more diluted suspensions. For example, based on the 10% suspension which had a count of 38,640 particles/mL, the 32 times more dilute suspension of 0.313% should have a calculated count of 1208 particles/mL. The actual count is 1280 particles/mL or 6% higher, possibly indicating that background counts become more important in the lower range.

A nonlinearity was also observed for the hemacytometer but in an opposite direction. For example, based on the 5% suspension which had a count of 25,000 cysts/mL, the 8 times more dilute suspension of 0.625% should have had a calculated count of 3125 cysts/mL. The actual count was 2040 cysts/mL or only 65% of the calculated amount, indicating that at low cysts concentration the counting of the cysts in the squares of the small volume of the counting chamber may miss actual cysts.

The coefficient of correlation between the particle counts and the dilution percentage was 0.96 for the Coulter Counter and 0.98 for the hemacytometer. The standard deviation using the Coulter Counter, however, was much lower than that of the hemacytometer. While the average coefficients of variation of the Coulter Counter was as low as 1.94%, it was as high as 74.2% for the hemacytometer indicating that the Coulter Counter is more precise. In Figure 11, the coefficient of variation as related to

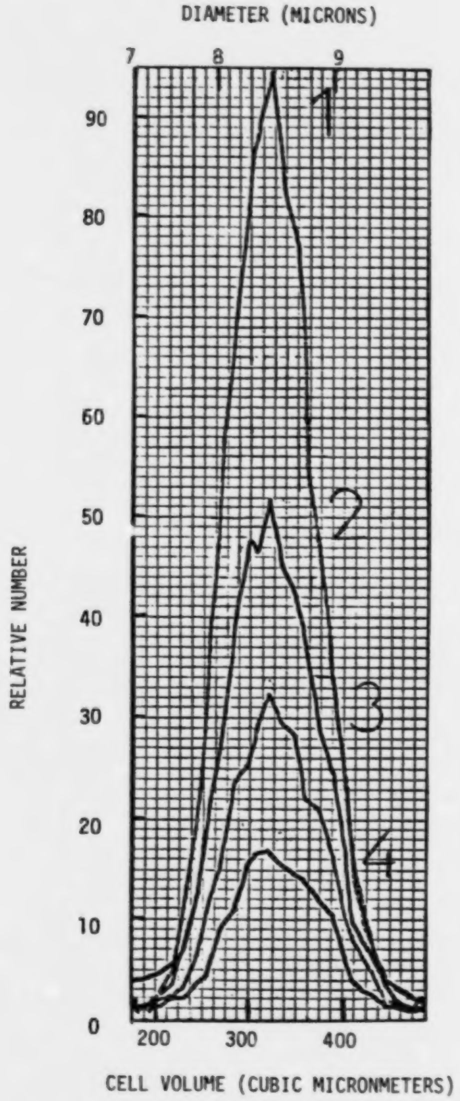


Figure 9. Size distribution of serially diluted Giardia suspension in distilled water at (1) 5%, (2) 2.5%, (3) 1.25% and (4) 0.625% of the stock solution.

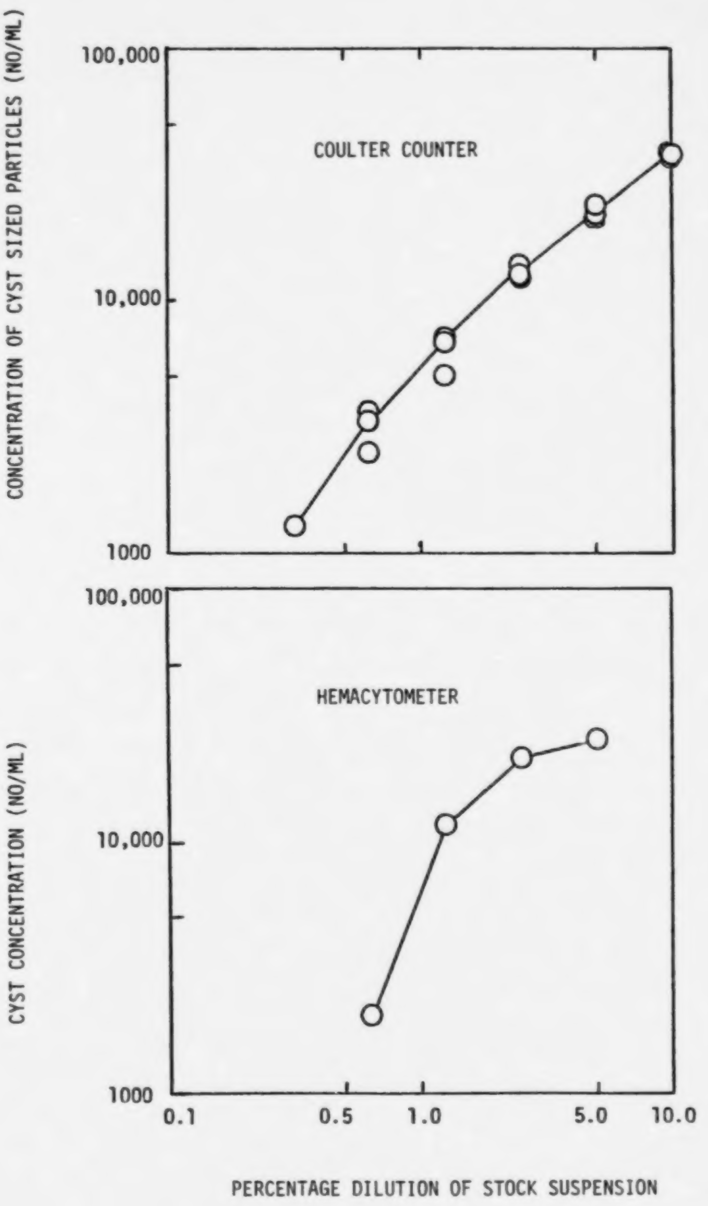


Figure 10. Linearity of two counting methods for enumerating Giardia cysts.

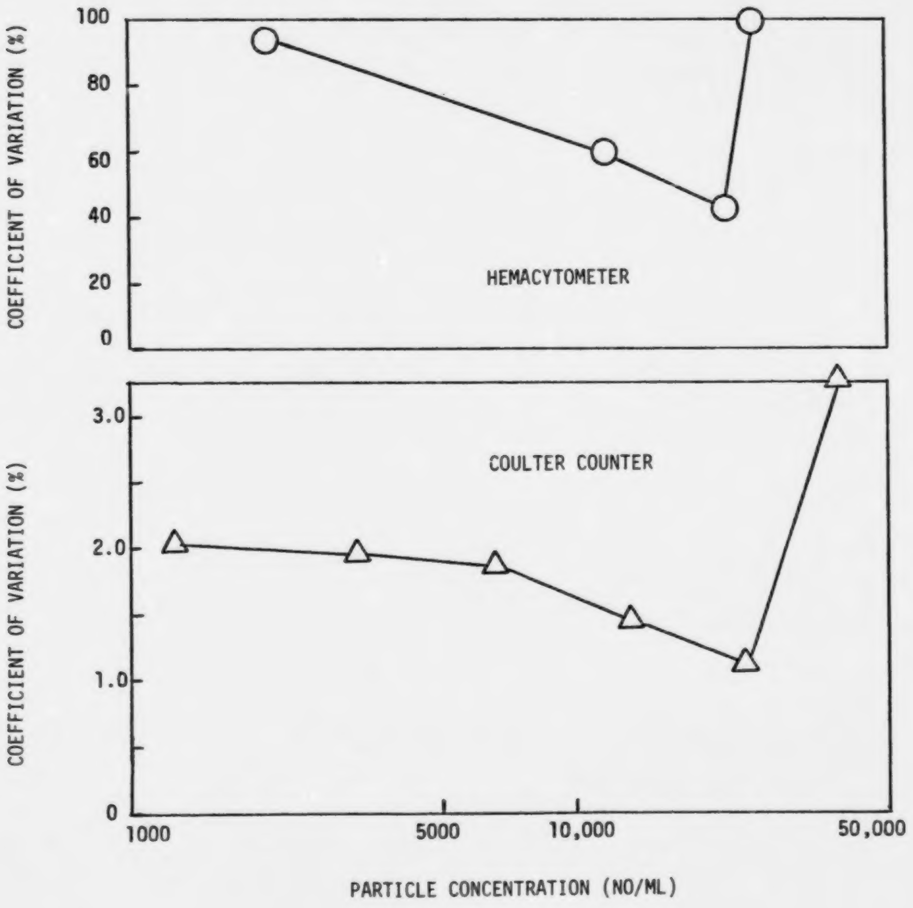


Figure 11. Coefficient of variation for two methods used for enumerating *Giardia* cysts.

the concentration of the cysts, shows a minimum around 20,000 cysts/mL for both methods, indicating that the most precise results are obtained at this concentration.

The lower detection limit of the Coulter Counter was lower than that of the hemacytometer. The minimum amount of particles that could be detected with the former method in 0.5 mL of solution was 250. The lower limit for the hemacytometer in 0.0001 mL of solution was 1 cyst. This indicates that the former method was 5000 times more sensitive. The above results therefore clearly indicate that the reproducibility and range of the Coulter Counter were greater than for the hemacytometer. However, the method was nonspecific for cysts and included other particles with the same size range as cysts.

Evaluation of 47mm Membrane Recovery Technique

An example of a recovery test using Lake Union water, one of the water sources available for the University of Washington pilot plant, spiked with cysts is shown in Figure 12. The counts in the size range of *Giardia* were 19,564 particles/mL before and 13,112 particles/mL after the recovery accompanied by a small apparent decrease in size of the particles. These counts were substantially above the 220 particles/mL background count of Lake Union water in the 8 to 12 um size range.

The average recoveries of the 5.0 um Millipore and Nuclepore membranes are shown in Figure 13. The Millipore membrane recovery using *Giardia* cysts in distilled water ranged from 55% to 90% with an average of 75.2%. The average recovery using Lake Union water was 77.8% using Millipore and 72.3% using Nuclepore. These results indicate no major differences between the membranes even though the former had a sponge-like structure and the latter had a pinpoint-hole structure.

Evaluation of 293 mm Membrane Recovery Technique

The average recovery of cysts at initial concentrations ranging from 10^3 cyst/mL to 10^5 cysts/mL measured with the hemacytometer was 20% (Figure 14) using the Millipore membrane and 85% with the Nuclepore membrane indicating that the membrane structure may have had an effect when using the 293 mm membrane. At concentrations below 1 cyst/mL the recoveries became highly variable due to the low number of cysts that could be enumerated. For example, the recovery of duplicate runs at 0.1 cyst/mL was 75% and 23%, respectively. The lower recovery using the 293 mm Millipore filter, as compared to the 47 mm unit, may be due to the greater difficulty of removing the entrapped cysts from the surface of the large membrane by agitation using the shaker bath.

Evaluation of Membrane Cassette Unit

The cassette unit was evaluated and showed a recovery of 0.44 to 2.34% (Table 1) which was lower than observed for the 293 mm membrane. The low

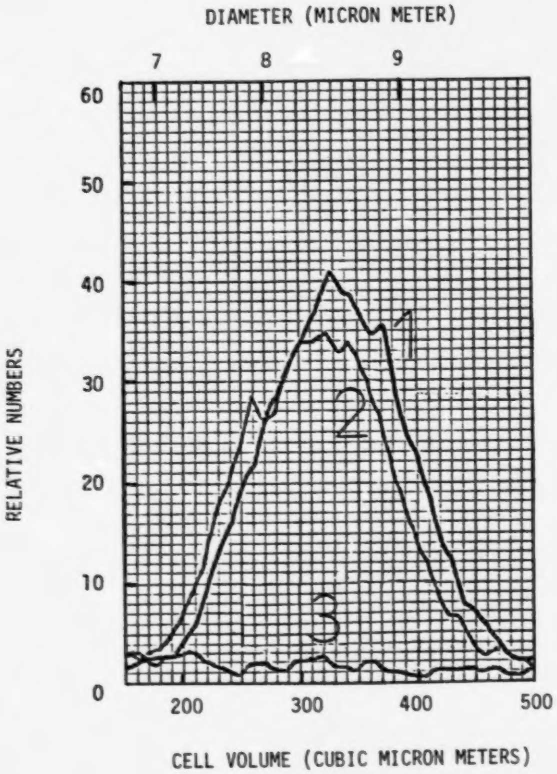


Figure 12. Results of 47mm diameter membrane filter recovery test using Lake Union water spiked with Giardia cysts. (1) Before recovery, (2) recovered cysts and (3) background counts.

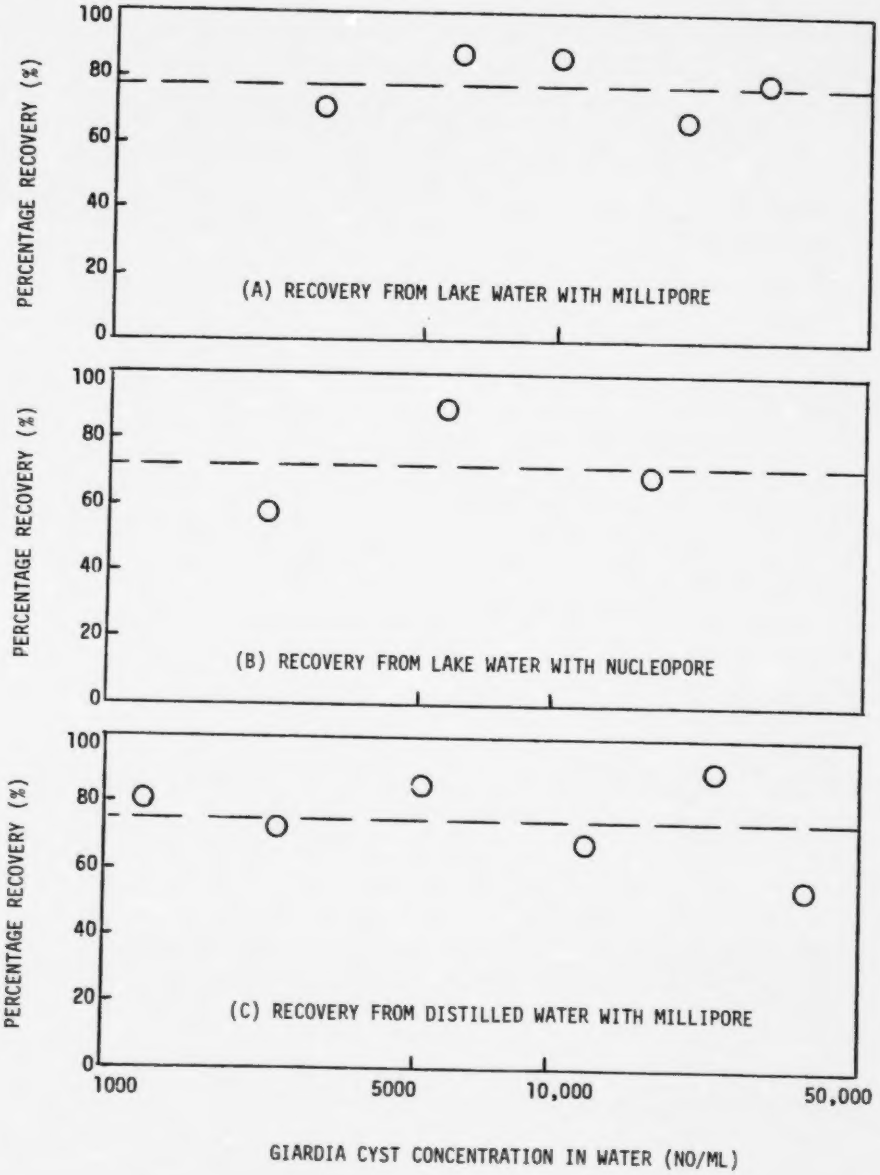


Figure 13. Percent recovery of Giardia cyst by different 47mm diameter membrane filters from two types of water.

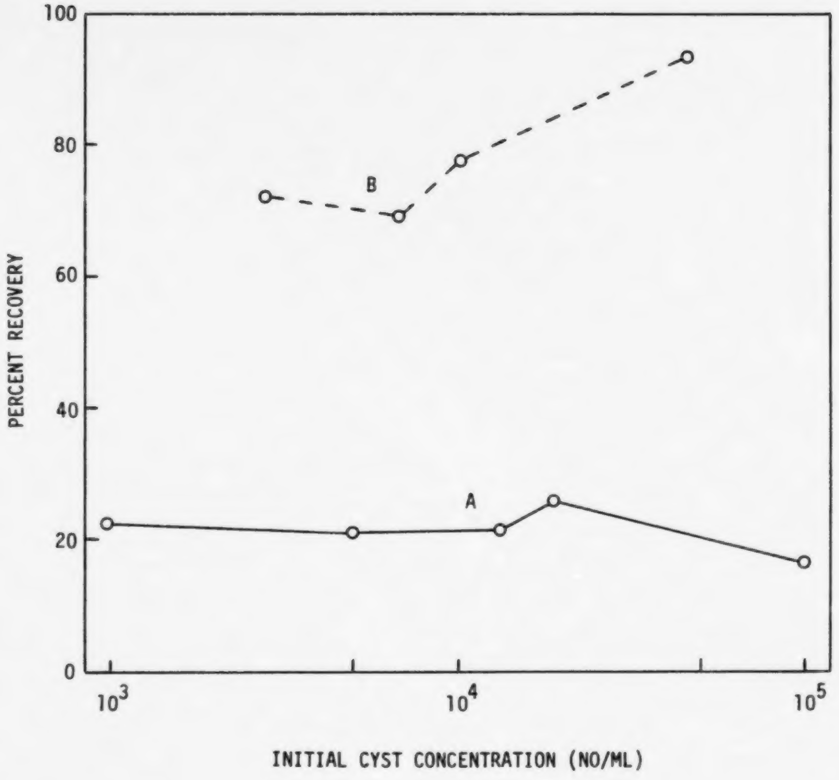


Figure 14. Percent recovery of cysts by 293mm diameter, 5 μm pore size membrane filters, (A) Millipore and (B) Nuclepore.

TABLE 1. SUMMARY OF LABORATORY RECOVERY RATES OF G. LAMBLIA CYSTS WITH MILLIPORE PELLICON CASSETTE UNIT

Initial concentration of stock (cysts/mL)	Volume of spike added to 20 L (mL)	Final concentration of membrane retentate and wash (cysts/mL)	Final volume of retentate and wash	Dilution factor	Equivalent retentate concentration (cysts/mL)	Recovery percentage
10,460	16	320	120	7.5	4,600	0.44
2,960	20	330	270	13.5	6,920	2.34
2,960	20	305	270	13.5	5,880	2.0
2,040	20	350	325	16.25	3,850	1.9
5,480	16	440	135	8.4	4,820	0.88

Mean Recovery Rate (n = 5) is 1.5 S.D. = 0.72

recovery may be due to entrapment of cysts in the mesh separating the stacked filters.

No cysts of G. lamblia were microscopically observed during the recovery tests at Ryderwood, although numerous diatoms and other protozoa were visible in the retentate sediment. Both retentate and wash were then examined for its particle size distribution with the Coulter Counter, resulting in 23 out of 560 particles/mL counted in the Giardia size range.

Zeta Potential of Cysts

The zeta potential values for the fixed Giardia lamblia cysts clearly show a decreasing potential at decreasing pH values (Tables 2 and 3). However, even at low pH values the cysts retain their negative charge (Figure 15). The Zeta potential was always more negative than -20 mv in the range of pH 5 to pH 10.

TESTING OF UNIVERSITY OF WASHINGTON PILOT PLANT

Unit process detention times for the 2.3 L/min (0.6 gpm) pilot plants were determined by addition of NaCl or dye. The tracer concentration was measured and plotted as a function of time to determine the retention time. A second plot on semilogarithmic coordinates gave information about the overall performance of the unit process reactor. The fraction of tracer remaining at a given time (1-F[t]) was plotted as a function of the ratio between the time of tracer measurement and theoretical retention time (t/T).

The actual retention time for the rapid mix was 2.1 min compared to 2.3 min as was estimated theoretically. The tank had a completely mixed flow regime as evidenced by the semilogarithmic plot (Figure 16). At the theoretical retention time T, 67% of the tracer had been displaced and only 19% remained at 1.5T.

The three flocculation tanks were studied individually and in series. By itself, each of the compartments behaved as a completely mixed reactor. However, as expected, with the tanks in series, the flow regime was approaching plug flow (Figure 17). Although not intended for application to stirred reactors, the relationship between retention time, dead space and flow regime developed by Rebhun and Argaman (1965) can provide useful information on flocculator performance. Applying this relationship to the tracer data, the three tanks in series were approximately 53% plug flow. At the theoretical retention time, 23.2 min, 64% of the tracer had been displaced and only 12% remained after one and one half times the theoretical retention time. The actual retention time was 17.3 min.

The dye testing of the sedimentation tank revealed that a fair amount of mixing was occurring throughout the tank (Figure 18). Only about 15% of the flow was plug flow. It was believed that the flow regime could be improved by constructing a better baffled inlet zone, although some of the

TABLE 2. ZETA POTENTIAL (ELECTROPHORETIC MOBILIGY) OF BUFFERED FORMALIN FIXED _GIARDIA LAMBLIA_ CYSTS AT VARYING PH VALUES AND CYST CONCENTRATIONS

Exp. #	Cyst Conc. (#/ml)	Spec. Conductance (microhmos)	pH	ZP($\bar{x}$) in mv. corrected	N	SD
1.	4×10^4	1,800	3.5	-17.4	6	5.3
2.	5.25×10^4	340	4.3	-14.4	8	7.0
3.	4×10^4	3,000	5.6	-24.7	6	3.6
4.	4×10^4	3,500	6.0	-31.3	14	8.4
5.	4×10^4	4,000	8.0	-33.8	6	10.9
6.	2.14×10^4	12,000	10.0	-39.2	4	9.0

*_Giardia_ suspensions used in #1, #3, #4, and #5 were from same stock suspension. #2 and #6 were from different stock suspension.

TABLE 3. ZETA POTENTIALS OF FIXED _GIARDIA LAMBLIA_ CYST SUSPENSION AT DIFFERENT PH VALUES

Specific Conductance (microhmos)	pH	ZP($\bar{x}$)	N	SD
340	3.8	-21.0	10	7.2
1,200	5.5	-25.5	10	5.6
1,600	7.5	-27.1	10	3.3
3.200	10.0	-37.3	10	4.3

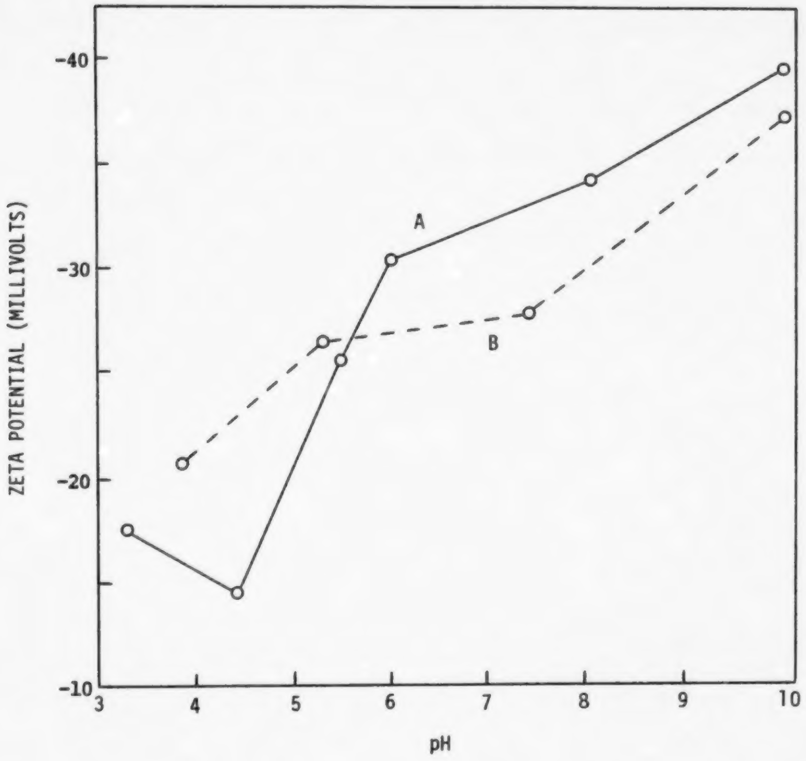


Figure 15. Effects of pH on the zeta potential of fixed G. lamblia cysts, (A) different suspensions and (B) same suspension.

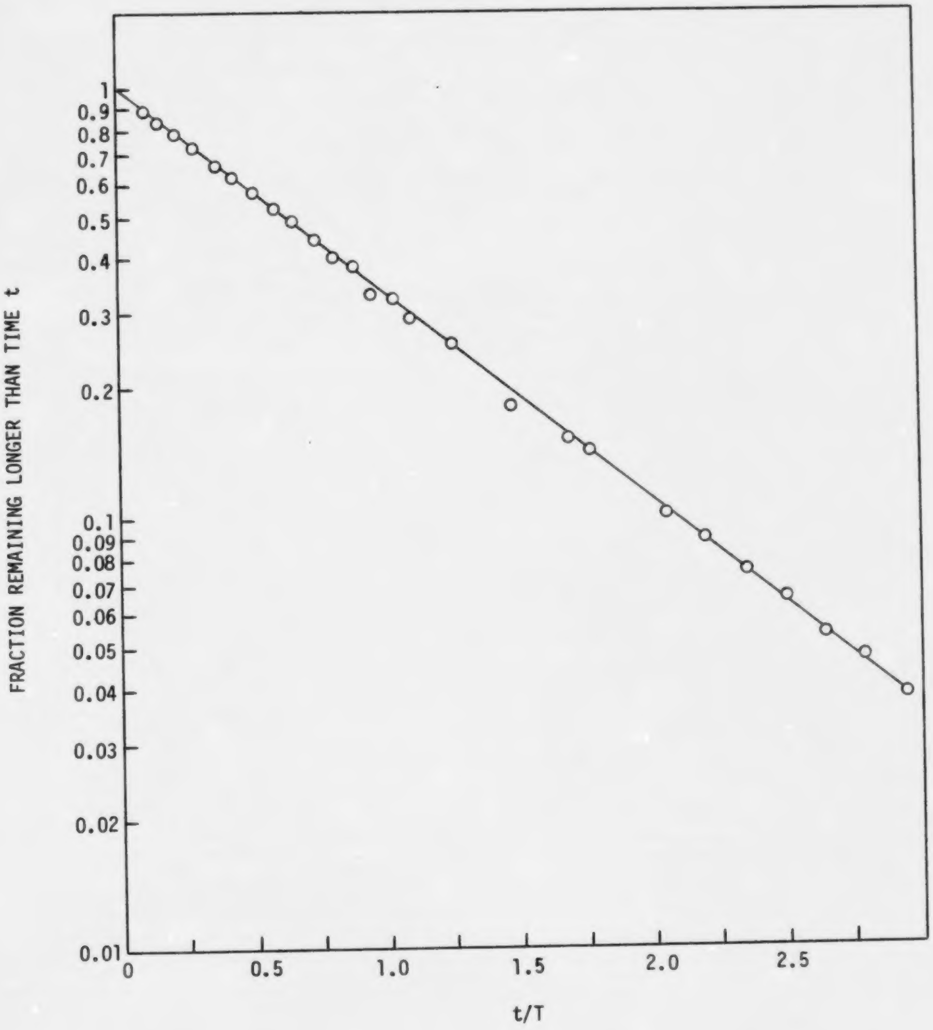


Figure 16. Tracer evaluation of the rapid mix tanks.

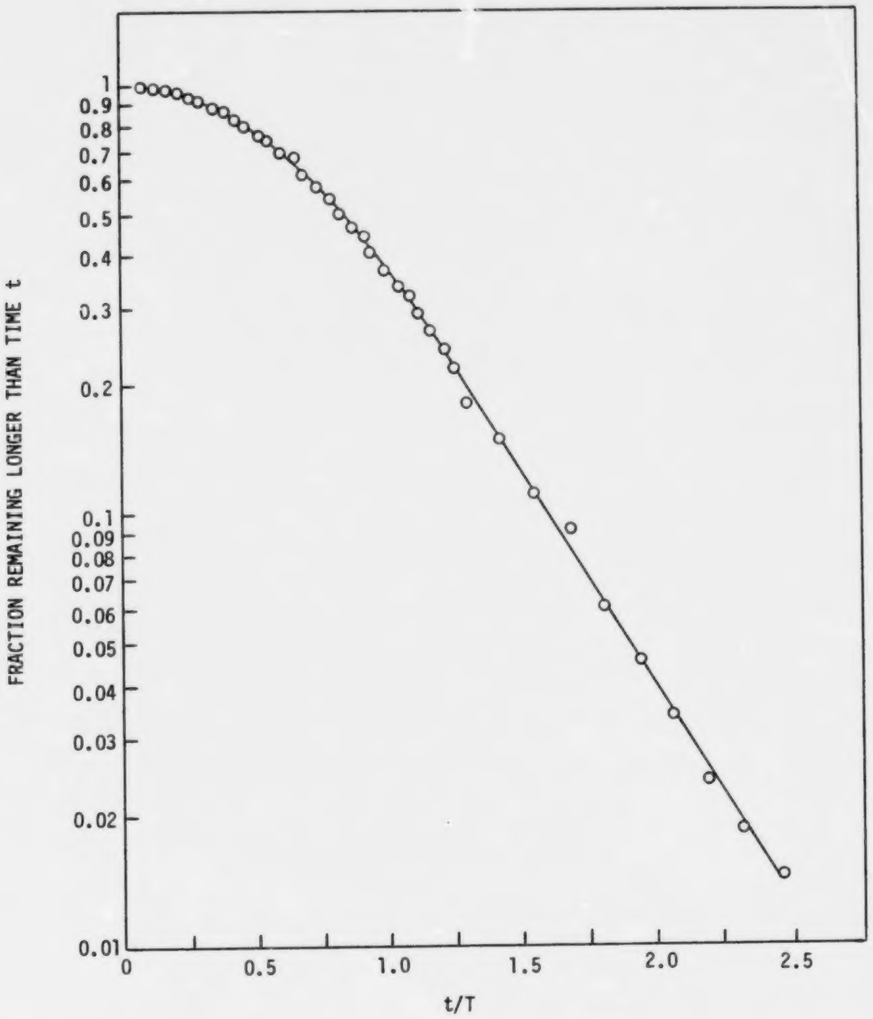


Figure 17. Tracer evaluation of the flocculation tanks.

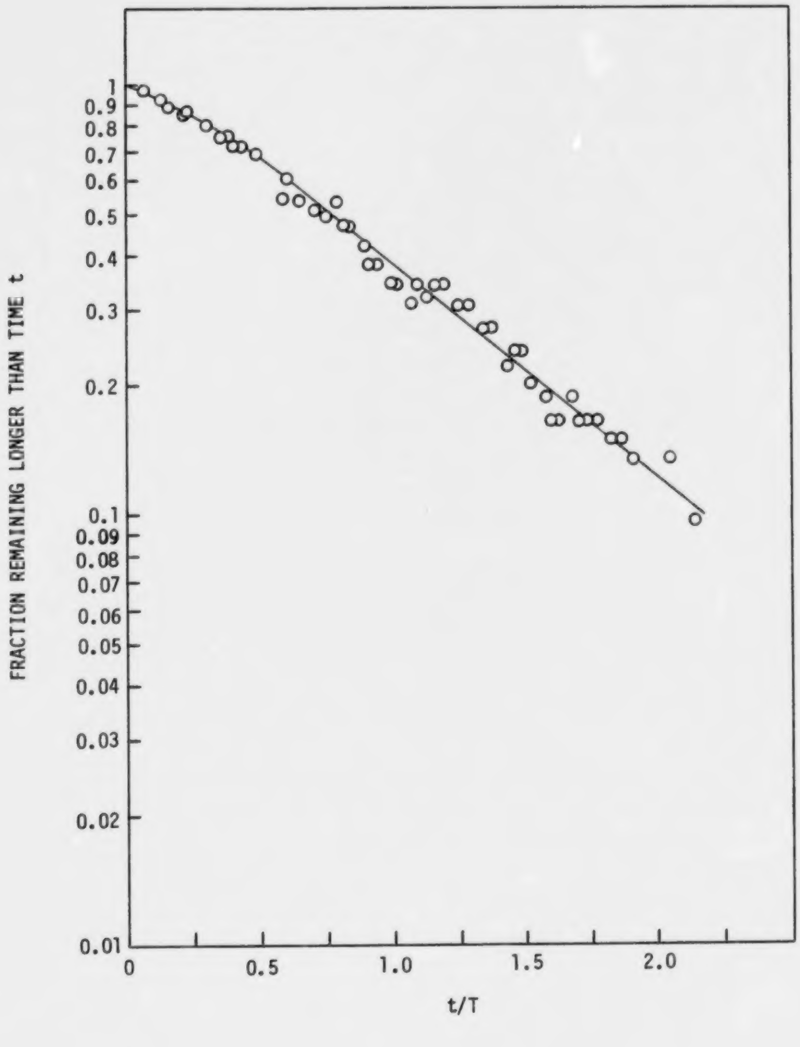


Figure 18. Tracer evaluation of the sedimentation tanks.

mixing was likely caused by density currents. The incoming water was around 10° C compared to an ambient temperature of 22° C. The theoretical retention time was estimated to 5.6 hrs, whereas the actual, as determined by the dye test, was 3.9 hrs. At the theoretical retention time, 38% of the dye remained in the tank and 12% was still left in the tank after two times the theoretical retention time. The tracer data indicated no dead space.

With the objective to determine the optimum speed of the flocculator paddles, a series of tests was performed with a 10 mg/L alum coagulant dosage and pH 6.7, adjusted by the addition of lime. The rapid mix speed was kept constant at 500 rpm throughout the tests. Following the selection of the mixing speed to be evaluated, the pilot plant was operated for 40 min. Samples were collected from each of the three flocculation compartments and allowed to settle in a jar for 30 min, at which time the turbidity of the settled water was determined. Based on these tests, a 22 rpm mixing speed was selected. The corresponding Gt and G values were calculated to be 49800 and 48 sec^{-1}, respectively. The sample collected from the first compartment with less than 6 min flocculation time did not settle well compared to the sample from the third compartment with 17.3 min flocculation.

TESTING OF COAGULATION/FILTRATION AND DIRECT FILTRATION AT UNIVERSITY OF WASHINGTON

Jar Tests

Batch tests with different alum dosages showed that the lowest turbidity after settling was obtained at a dosage of 8 to 12 mg/L using 1 L beakers.

Continuous Testing with Giardia Spiking - Conventional Treatment

The first seven runs were made using the coagulation/sedimentation unit followed by filtration. Runs made thereafter were direct filtration runs bypassing the sedimentation unit.

The results of the single spike of Giardia cysts (Run 1), added to the first flocculation compartment of unit A are summarized in Table 4. This run was performed primarily to determine removal of cyst-sized particulates, cysts and turbidity by both flocculation, sedimentation and filtration. No cysts were detected in the filter effluent while high removals of particles in the cyst-sized range were observed together with high turbidity removals.

The turbidity removal by the filter was more than 96% and the run was terminated after 80 hrs due to high headloss in Filters B and C (Figure 6). In this figure and in later figures showing University of Washington pilot plant filter run data, the data points identified as overflow are for water applied to the filters.

TABLE 4. RESULTS OF A SINGLE DOSE SPIKE OF GIARDIA CYSTS INTO FLOCCULATION COMPARTMENT OF PILOT PLANT - RUN #1

Sampling point	Sampling time (hrs. after spike)	Particulate concentration (no/mL)	Particulate removal[γ] in preceeding process (%)	No. cysts found in 201. concentrate	Turbidity (NTU)	Turbidity removal[δ] in preceeding process (%)	Headloss (ft)
Tap water -- plant influent	before run	2017[ϕ]	---	---	0.44	---	--
1. overflow from sedimentation basin	1.5	20.01	99.0	0	0.58	-31	--
2. filter C effluent	1.5	.348	98.3	0	.02	96.6	6
3. filter C effluent	4.5	3.7	81.5	0	.022	96.2	7.4

*Approximately 170,000 cysts added as single dose to first flocculation compartment at A_1.

[ϕ] Concentration of Giardia size particulates in influent tap water is calculated from Coulter enumeration of 7.5 L. water passed through 5.0μm 293mm Nuclepore filter and processed as in section 2.4.

[γ] Particulate removal is calculated as 100 - (concentration out/concentration in). Initial concentration of particulates for filter efficiency is Giardia size particulate concentration in overflow weir. Initial concentration of particulates for sedimentation efficency is Giardia size particulate concentration of influent tap water.

[δ] Turbidity removal is calculated similarly to that of particulate removal.

A continuous addition of Giardia cysts (Run 2) added to the chemical intake port is summarized in Table 5. Cyst-sized particle removal is defined as removal of 8 to 12 µm particulates as observed on the Coulter Counter. Particulate removal was 99.0% for coagulation/sedimentation and 90% for filtration. Mean turbidity removal by filtration as monitored periodically from Filters B and C effluents was 87% and 92%, respectively. Total cyst-sized particle removal for the entire treatment train was 99.9%.

Three cysts were observed by microscopic examination in two different effluent samples; i.e., at the beginning and at the end of the run. Estimated removal of cysts by coagulation/sedimentation and filtration was 99.8% or above at an influent concentration of 225 cysts/L and 0.05 cysts/L in the effluent.

After a ripening period a high quality effluent was produced while the headloss showed an approximately linear increase with time.

In the third run Giardia cysts were added directly to the dual media Filters B and C. Cyst-sized particulate removal by filtration throughout the run averaged 74% and 62% for B and C, respectively. Poor floc formation and subsequent low turbidity removal were probably a result of inadequate lime feeding. Cyst-sized particulate removal by sedimentation was greater than 99% in spite of a low turbidity removal of 44% (Table 6).

When adding 984 cysts/L at the influent of Filter B in Run 3, 8 cyst/20 L were recovered in the effluent, corresponding to the 99.96% removal. When 622 cysts/L were added at the influent of Filter C, the effluent concentration was 5 cysts/20 L, which is also a 99.96% removal.

The influent and effluent quality data of Run 4 (low pH) showed an average cyst-sized particle removal of 99.9%, while the turbidity removal was 85%. The 1093 cysts/L in the influent of Filter C correspond with a worst effluent concentration of 0.6 cyst/L in the effluent, which was a 99.95% removal.

The effluent turbidity of the fourth filter run is shown in Figure 19, together with the cyst effluent concentration. The increase of headloss with time was primarily accounted for in the top 5 cm (2 in) of the filters.

Run 5, conducted at high pH (7.2), showed an average particle removal of 80% and turbidity removal of 76%. Cysts were added to Filter C only, at a concentration of 23 cysts/L. The filter effluent concentration was 0.75 cysts/L corresponding to a 96.74% removal. The higher pH resulted in a slow rate of headloss buildup, but uniform throughout the depth of the filter.

The effluent quality during Run 6 with no pH adjustment (pH 6.4) showed a significant improvement over Run 5 with respect to turbidity and cyst-sized particle removals (Figures 20 and 21). The cyst removal, however, was essentially the same. Of the 30 cysts/L added to the influent of Filter C, 1 cyst/L was recovered from the effluent or a 96.67% removal. The gradual headloss buildup was primarily restricted to the top one-third of the filter.

TABLE 5. RESULTS OF CONTINUOUS SPIKE OF GIARDIA CYSTS INTO PILOT PLANT - RUN #2

Sampling point	Sampling time (hrs. after spike)	Particulate concentration (no/mL)	Particulate removal in preceeding process (%)	No. cysts found in 20 L. concentrate*	Turbidity (NTU)	Turbidity removal in preceeding process (%)	Headloss (ft)
Tap water	before run	2017	---	---	.50	---	--
1. overflow from sedimentation basin	2	10.4	99.5	0	0.2	60	--
2. filter C effluent	2	0.54	94.8	1	.037	81.5	1.7
3. filter B effluent	7	1.33	87.3	0	.038	87	2.2
4. filter C effluent	7	1.22	88.3	0	.026	87	2.0
5. filter B effluent	23	1.34	87.1	0	.03	85	3.1
6. filter C effluent	23	0.47	95.5	0	.018	91	3.3
7. filter B effluent	47	0.78	92.5	0	.028	86	4.2
8. filter C effluent	47	1.04	90.0	0	.019	90.5	4.7
9. filter B effluent	98	2.02	80.6	1	.03	85	5.4
10. filter C effluent	98	.099	90.5	1	.018	91	6.0
11. backwash sample from filter C at end of run	100	4650	---	720[γ]	--	---	--
12. sedimentation basin after run	100	4.7×10^5[δ]	---	2.0×10^6[Δ]	--	---	--

*An estimate of Giardia cysts per 20 L. sample was calculated by microscopic examination of 0.16 mL of sediment from 20 ml concentrate as recovered in section 2-4. Final number is extrapolation from estimate of sediment volume (approximately 0.20 mL).

[γ] An estimate of the number of cysts trapped by the dual media filters was calculated by microscopically examining the sediment from 1 liter of backwash water recovered as in section 2-4. Six cysts in approximately 0.04 mL of sediment were counted (of a total of 0.25 mL sediment). Extrapolation to 20 L. of backwash results in figure given. Removal of Giardia cysts by sedimentation can be crudely estimated by dividing the number of cysts in the sedimentation basin by the sum of the number of cysts in the backwash and the sedimentation basin.

[δ] An estimate of total particulates/ml removal by flocculation-sedimentation during the entire run was calculated by siphoning 4 L. (representating 8% of the total surface area) of the settled floc at the bottom of the sedimentation basin and enumerating by the Coulter method.

[Δ] An estimate of the total of cysts removed by flocculation-sedimentation was determined by hemacytometer counts of approximately 0.25 mL of sediment from 10 mL of floc centrifuged.

TABLE 6. RESULTS OF CONTINUOUS SPIKE OF GIARDIA CYSTS INTO PILOT PLANT - RUN #3

Sampling point	Sampling time (hrs. after spike)	Particulate concentration (no/mL)	Particulate removal in preceeding process (%)	No. cysts found in 20L. concentrate	Turbidity (NTU)	Turbidity removal in preceeding process (%)	Headloss (ft)
Tap water	before run	2017	---	---	0.5	---	--
1. overflow from sedimentation basin	2	3.78	99.8	cysts fed directly to filters	.28	44	--
2. filter B effluent	2	1.3	65.6	8	.12	57.1	.7
3. filter C effluent	2	2.5	34.0	2	.12	57.1	.83
4. filter B effluent	7.5	0.81	78.6	1	.165	41	.92
5. filter C effluent	7.5	0.88	76.7	5	.17	39	1.1
6. overflow	16.5	16.1	99.2	---	.35	30	--
7. filter B effluent	16.5	3.5	78.3	8	.2	42.8	1.2
8. filter C effluent	16.5	3.92	75.7	3	.2	42.8	1.25
9. backwash sample from filter C	22	1840	---	$1.2 \times 10^{5\Delta}$	---	---	--

*Estimate of cysts calculated

$^{\Delta}$An estimate of the total number of cysts in backwash sample was calculated by extrapolating hemacytometer counts of cysts in a total 0.25 mL of backwash sediment from 1 liter filtered through 5.0μm Nuclepore filter and processed as in section 2-4.

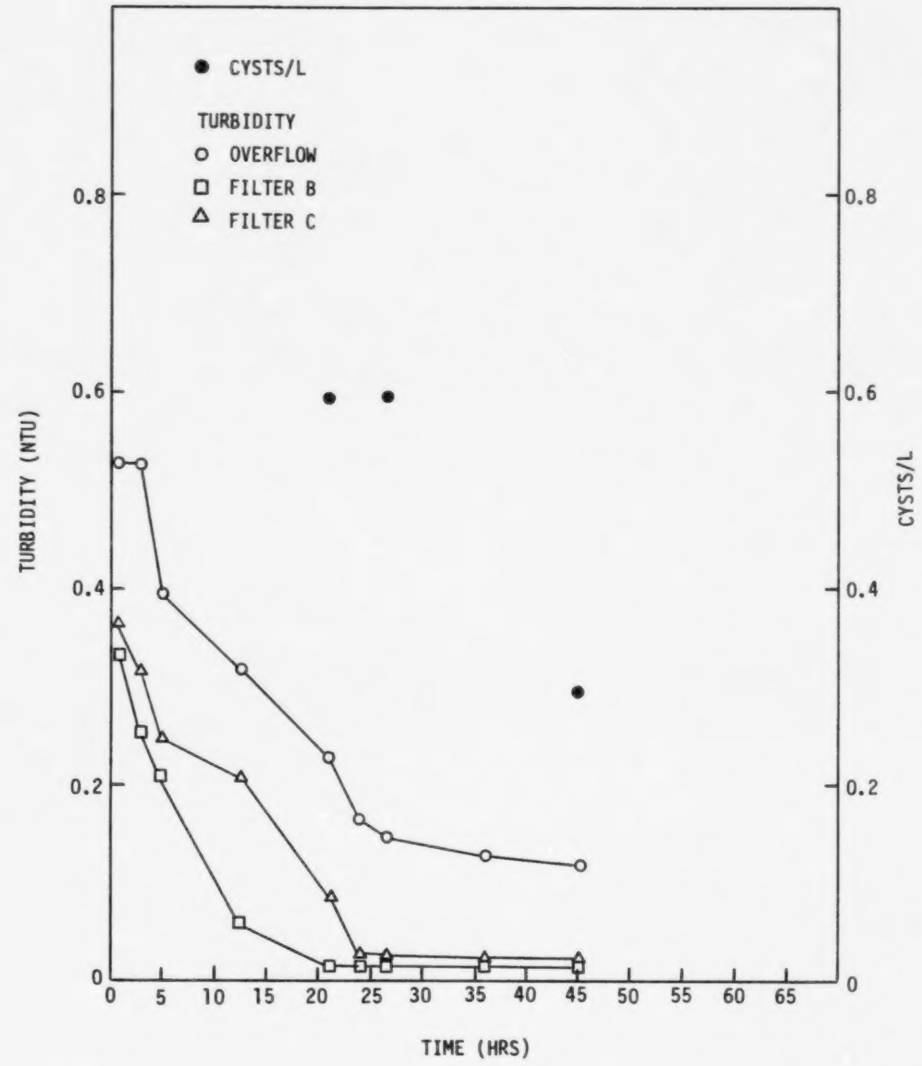


Figure 19. Turbidity in filter influent and effluent of Run No. 4.

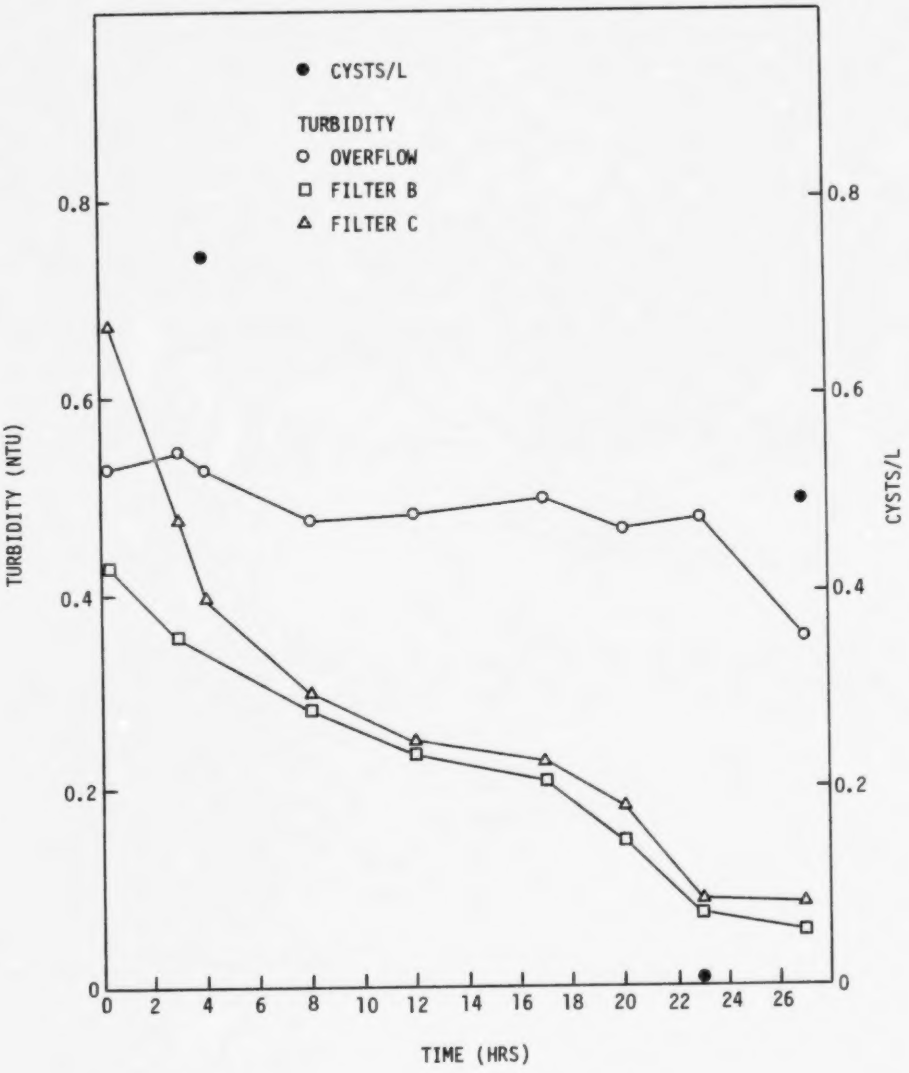


Figure 20. Turbidity in filter influent and effluent of Run No. 5.

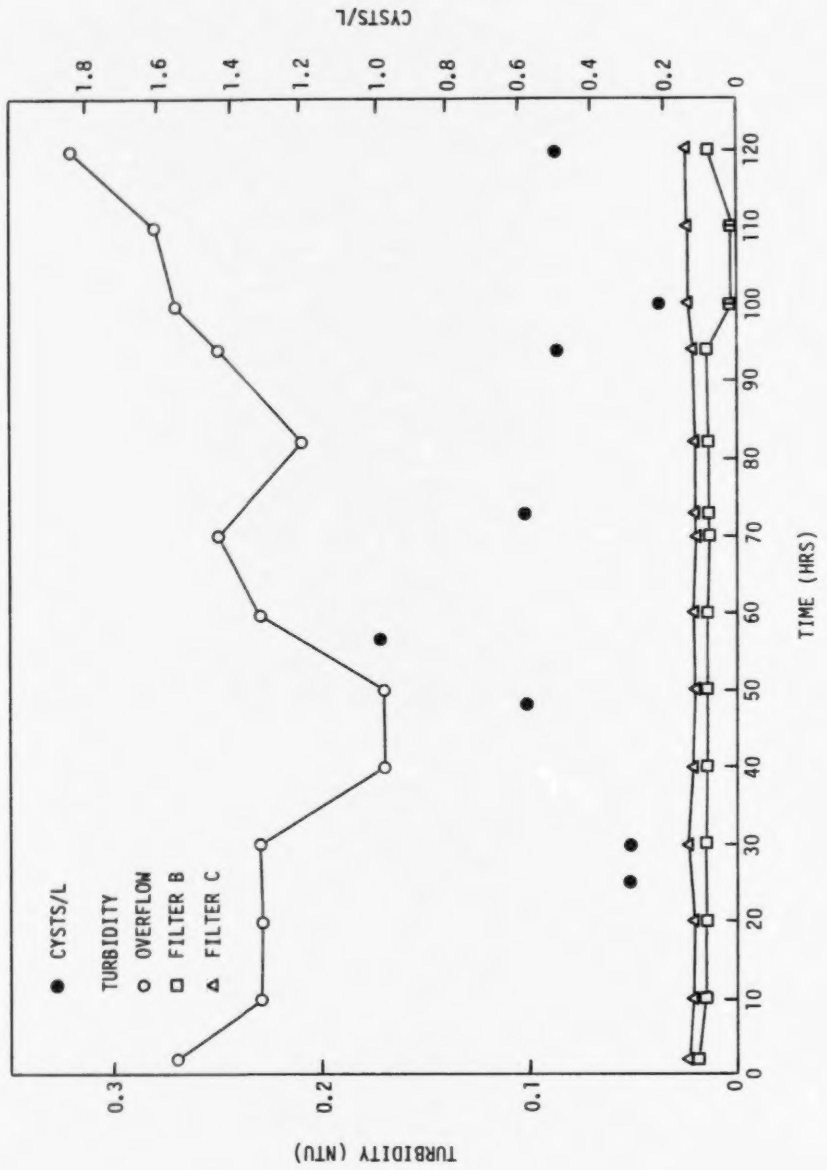


Figure 21. Turbidity in filter influent and effluent of Run No. 6.

Because of high pH (7.2), the seventh filter run showed a lower turbidity removal (Figure 22) than earlier runs with the same coagulant dosage (10 mg/L) and the pH ranging from 6.3 to 6.7. The largest increase in headloss developed at the anthracite/sand interface.

The results summarized in Table 7 generally show higher cyst removals at high spiking levels than at low spiking levels. This can be explained by the inherent limitations in the cyst enumeration technique. Low levels are difficult to determine accurately. Therefore, the investigator may sometimes have to settle for an upper boundary value which often will overestimate the number of cysts in the sample and underestimate the removal.

Direct Filtration at University of Washington Pilot Plant

This subsequent part of the study was devoted to the evaluation of the 8 to 12 um particle and turbidity removal efficiency of the University of Washington pilot unit. The unit was operated in the direct filtration mode treating chlorinated unfiltered Tolt Reservoir water.

The filter runs were conducted at different alum dosages, pH values and flow rates. The main parameters measured during the testings were: removal of particles in the *Giardia* size range, turbidity removal, length of filter run, headloss buildup at different depths in the filter and particle distribution at different filter depths.

The effect of alum dosage on the filtration efficiency is shown in Figures 23 and 24 for Filters B and C operated under identical conditions. Data were collected both at low (5.5 m/hr [2.3 gpm/ft^2] and 6.0 m/hr [2.5 gpm^2]) and high (9.6 m/hr [3.9 gpm/ft^2] and 13.5 m/hr [5.6 gpm/ft^2]) filtration rates for identical Filters B and C. The filter runs without any alum addition showed a 59% cyst-sized particle removal efficiency and 10% turbidity removal. The turbidity removal reached a maximum plateau at a 10 mg/L alum dosage while the particle removal did not increase further above 7 mg/L. The data of the University of Washington pilot plant show that particle removal exceeds turbidity removal below a dosage of 10 mg/L alum, possibly due to the inability of the filters to trap the small particles causing the turbidity, while still retaining the cyst-sized particles. Dosages above 15 mg/L greatly shorten the filter run and decrease the removal efficiency. Increasing the flow rate in the alum dosing range of 15 to 30 mg/L resulted in a slightly lower particle removal. The direct filtration runs at the high flow rate were less consistent than at the low flow rate and the run at 17 mg/L alum and 13.5 m/hr (5.6 gpm/ft^2) showed an unexpected lower removal efficiency and greater rate of headloss buildup.

The pH was also of major importance in the particulate removal and highest removals were observed at pH 6.5 and within a range of 5.6 to 7.0

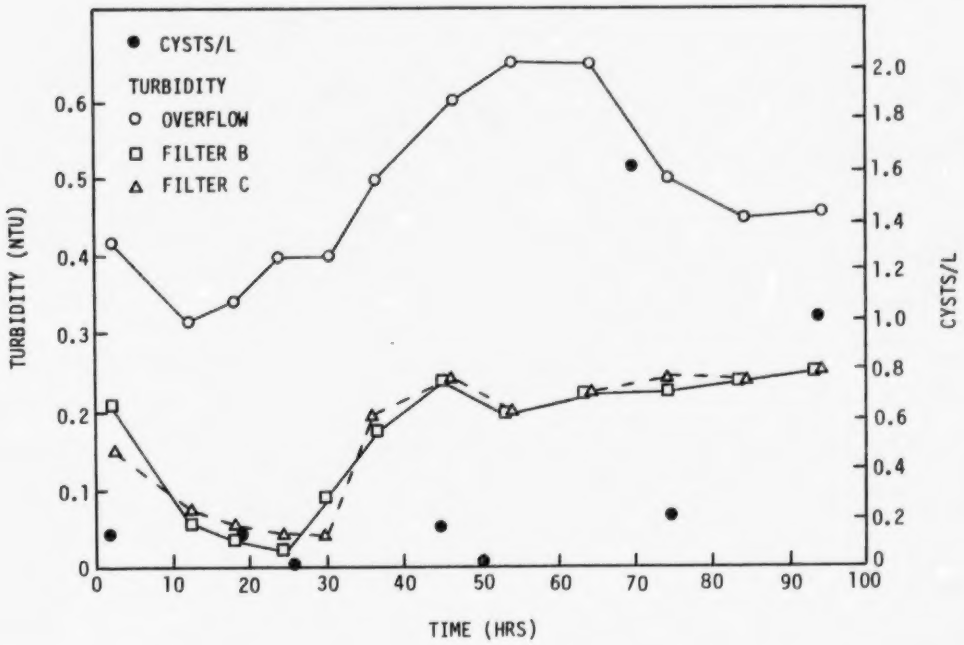


Figure 22. Turbidity in filter influent and effluent of Run No. 7.

Table 7. Performance of each filter run with cysts added directly to filter

Run No.		Cyst Dosage	% Cyst-sized Particle Removal	% Cyst Removal
3	6.7	984/1 (B) 622/1 (C)	93.1	99.96
4	6.3 (no lime)	1093/1 (C)	91.5	99.95
5	7.2 (high lime)	23/1 (C)	30.6	96.74
6	6.4 (no lime)	30/1 (C)	95.6	96.67
7	7.2 (high lime, low spiking level)	2.3/1 (C)	95.2	30.4

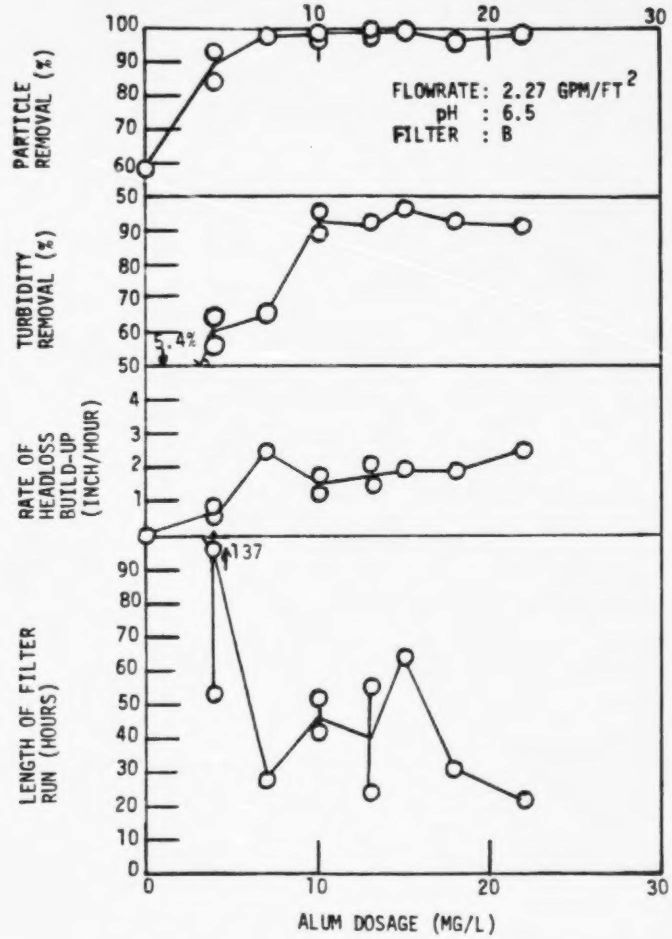


Figure 23. Effect of alum dosage on direct filtration process, Filter B.

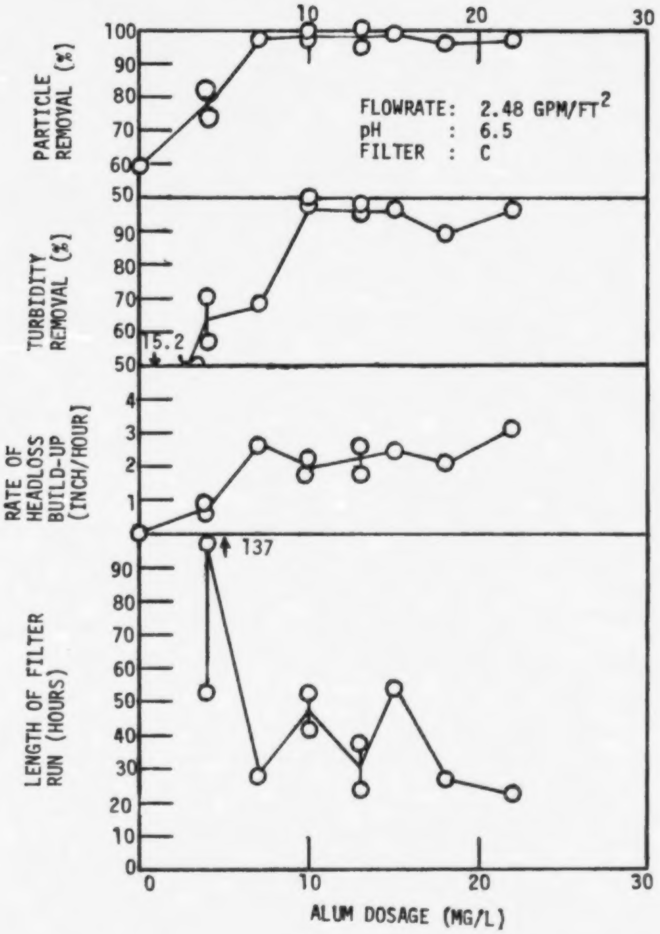


Figure 24. Effect of alum dosage on direct filtration process, Filter C.

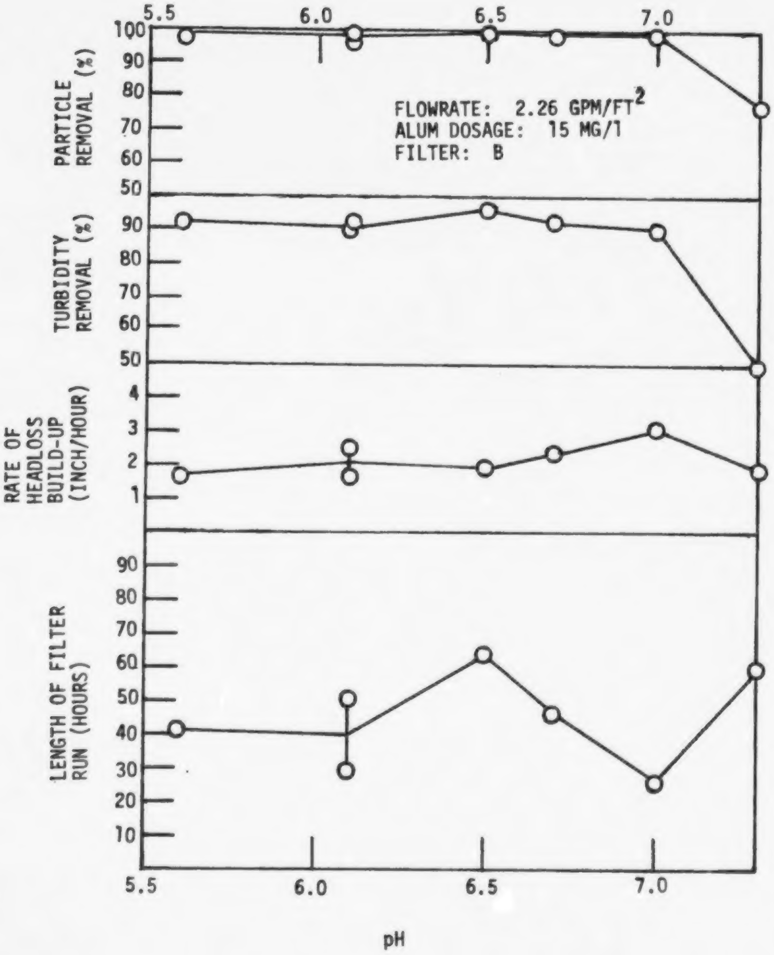


Figure 25. Effect of pH on direct filtration performance, Filter B.

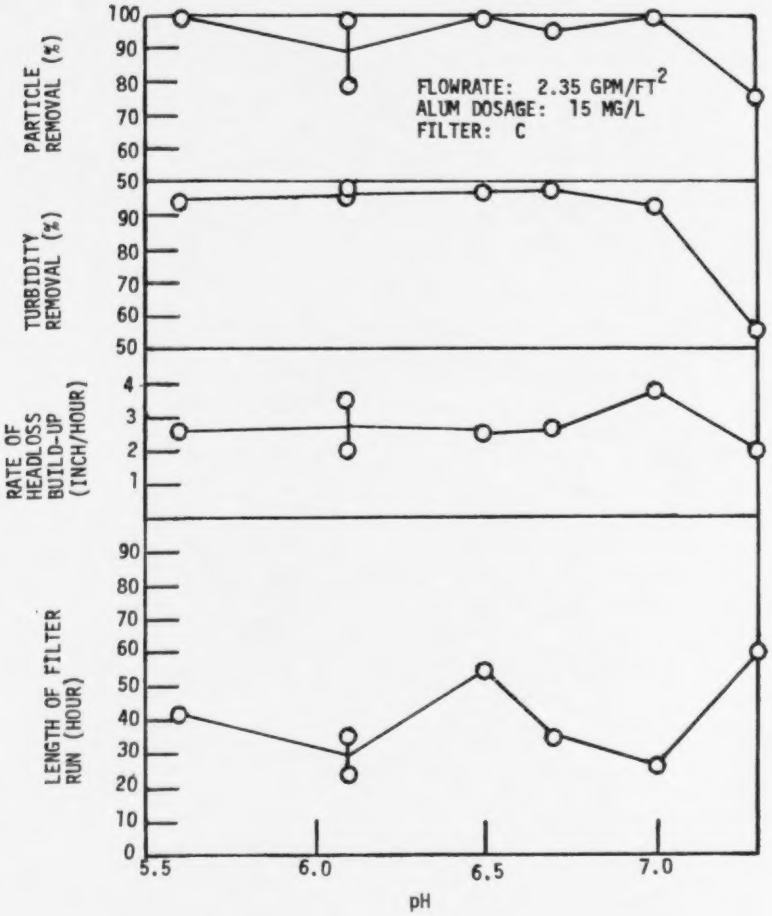


Figure 26. Effect of pH on direct filtration performance, Filter C.

(Figures 25, 26) using a dosage of 15 mg/L. Doubling of the flow rate resulted in a slightly lower particle removal but did not affect the turbidity removal. A pH increase from 6.5 to 7.0 resulted in a greater rate of headloss buildup at the higher flow rate than at the lower flow rate. Addition of soda ash was required to counteract the pH decrease resulting from the alum addition. A typical dosage of 7 mg/L soda ash was required to maintain the pH at 6.5 when using 15 mg/L alum. High rate runs generally produced poorer quality water, and the quality deteriorated even further when the pH was changed at the high filtration rates. The results in Figure 27 show that when the pH was temporarily changed from 6.5 to 6.8 the effluent turbidity increased from 0.12 to 0.65 NTU in Filter B and from 0.11 to 0.33 NTU in Filter C operated in an identical fashion. The influent turbidity was 0.90 NTU. The lower efficiency was also reflected in the decreased rate of headloss buildup.

Increasing the flow rate at a dosage of 15 mg/L alum and pH 6.5 resulted in a gradual decrease of turbidity and particle removal. Turbidity removal greatly decreased above 17.1 m/hr (7.0 gpm/ft^2) in Filter B, and particle removal decreased substantially above 12.9 m/hr (5.3 gpm/ft^2) in Filter C. The longest filter runs were observed below a flow rate of 6.1 m/hr (2.5 gpm/ft^2). Selecting flow rates above 17.6 m/hr (7.2 gpm/ft^2) resulted in a sharp increase in the rate of headloss buildup. The length of the theoretical water column that could be passed through the filter before backwashing showed a pattern parallel to that of the length of the filter run indicating that substantially longer runs were obtained below a flow rate of 6.1 m/hr (2.5 gpm/ft^2) (Figures 28 and 29).

Samples were carefully withdrawn from the three way valves at each of the headloss ports at different times during the the high flow rate filter runs. The results in Figure 30 show that the cyst-sized particles were gradually filling the voids in the upper parts of the filter. Thus a relatively sharp downward moving front existed between the filled voids and the empty pore spaces. The data indicate that at the 15 mg/L alum dosage the particle concentration in the upper portion of the filter was about two-fold higher than in the influent due to its accumulation and flocculation/sedimentation. This increase was about ten-fold at 20 mg/L alum and about fifteen-fold at the 30 mg/L dosage. Some differences were noted between the last two sampling ports after the filter; i.e., the port below the screen containing the filter media and the last port after passage of the effluent through the turbidity meters. The last port gave lower particle counts at the 30 mg/L alum run, possibly indicating some particle settling or attachment to the effluent tubing.

It was concluded that optimum operation of direct filtration was achieved at an alum dosage of 10 mg/L alum, and a pH of 6.5. The longest filter runs were obtained at a rate below 6.1 m/hr (2.5 gpm/ft^2). Sudden operational changes such as a pH increase caused a rapid effluent deterioration with respect to turbidity and cyst-sized particles. Quality deterioration was more pronounced when the conditions were changed rapidly as opposed to gradually.

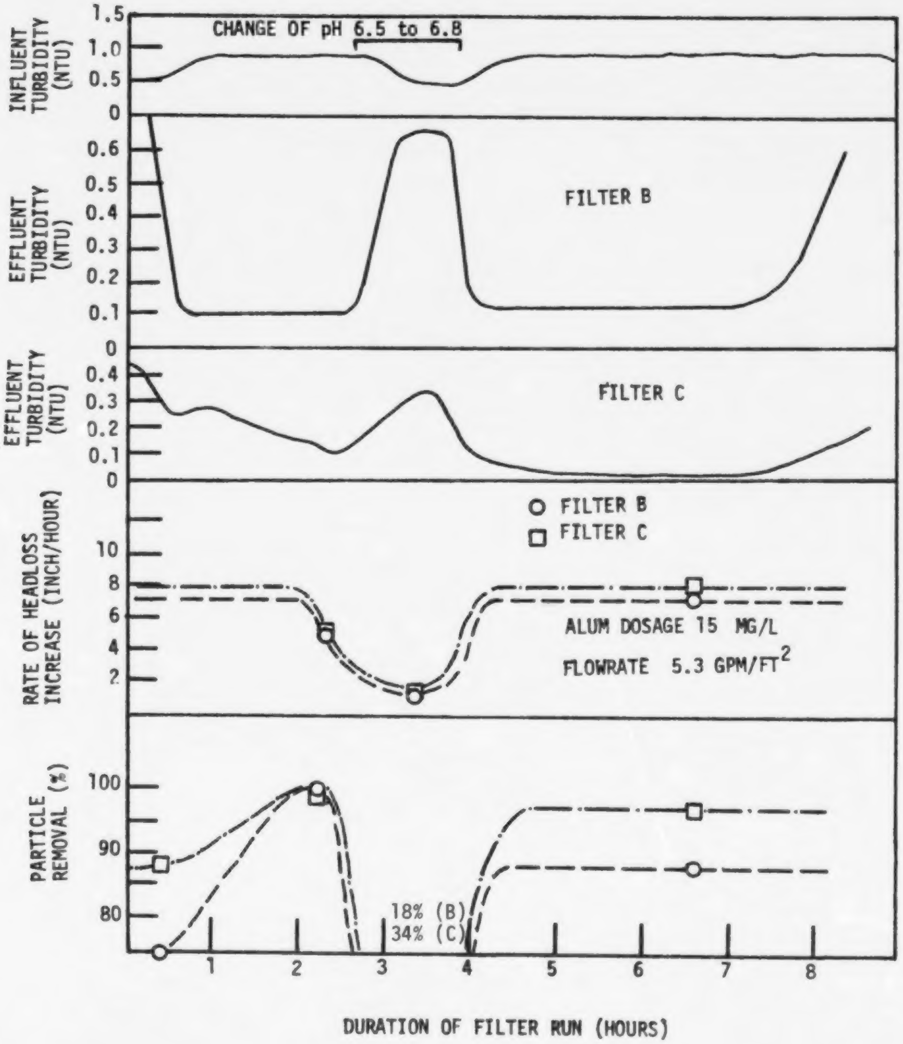


Figure 27. Effect of pH increase on filter performance.

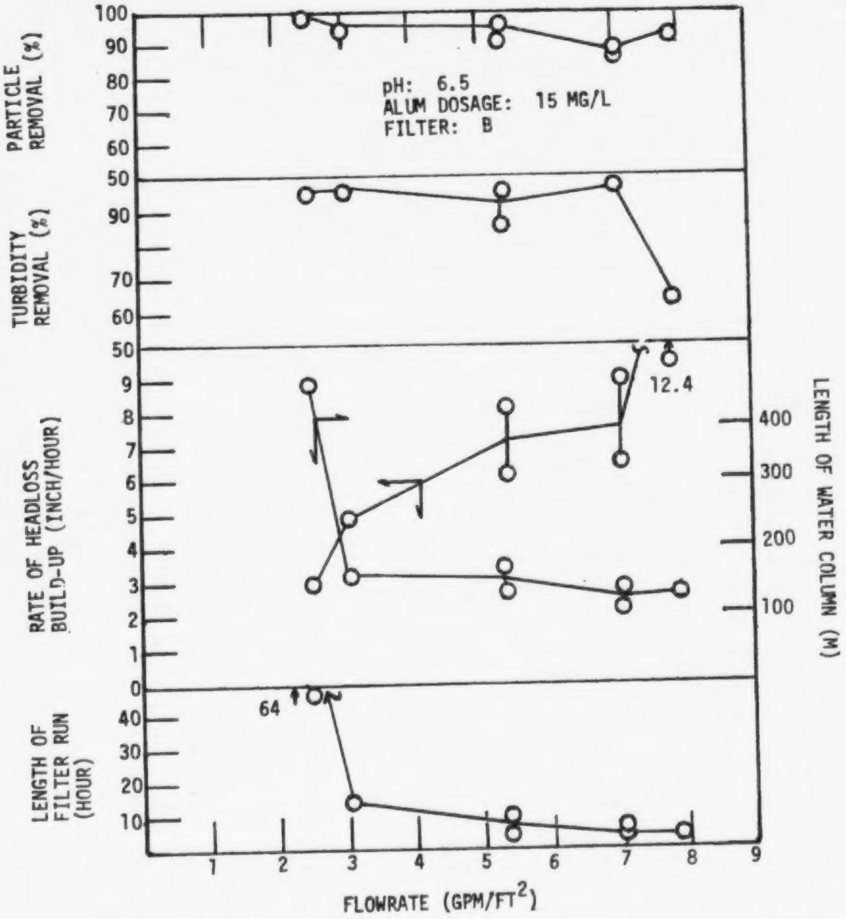


Figure 28. Effect of flowrate on direct filtration efficiency, Filter B.

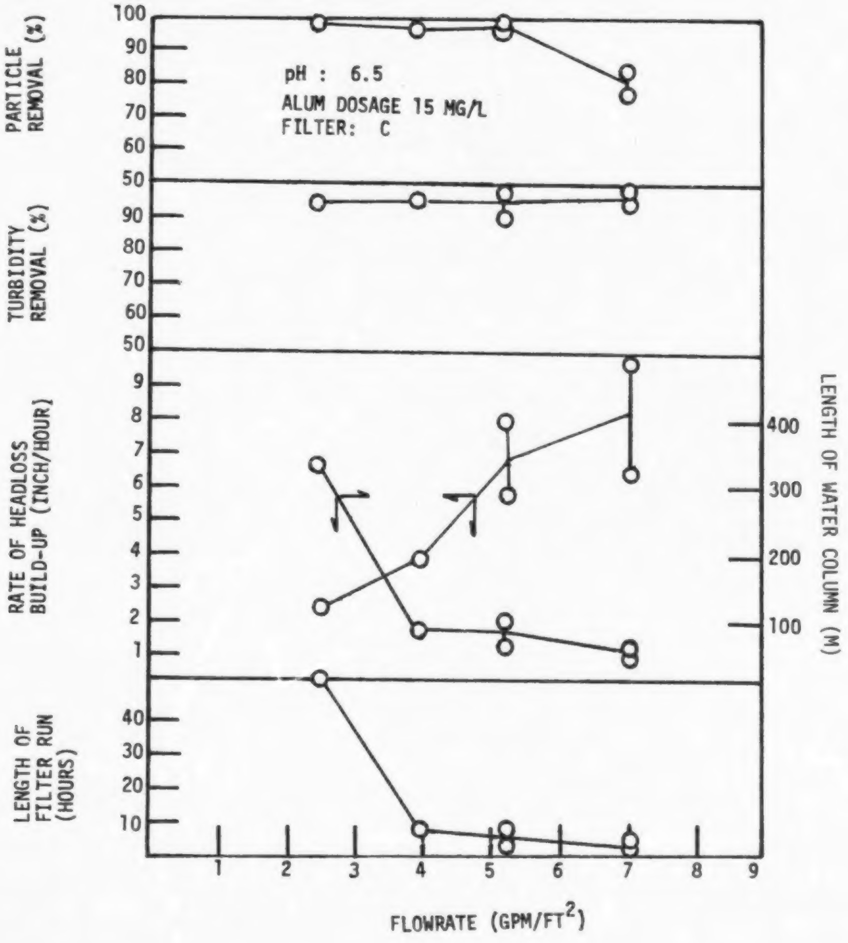


Figure 29. Effect of flowrate on direct filtration efficiency, Filter C.

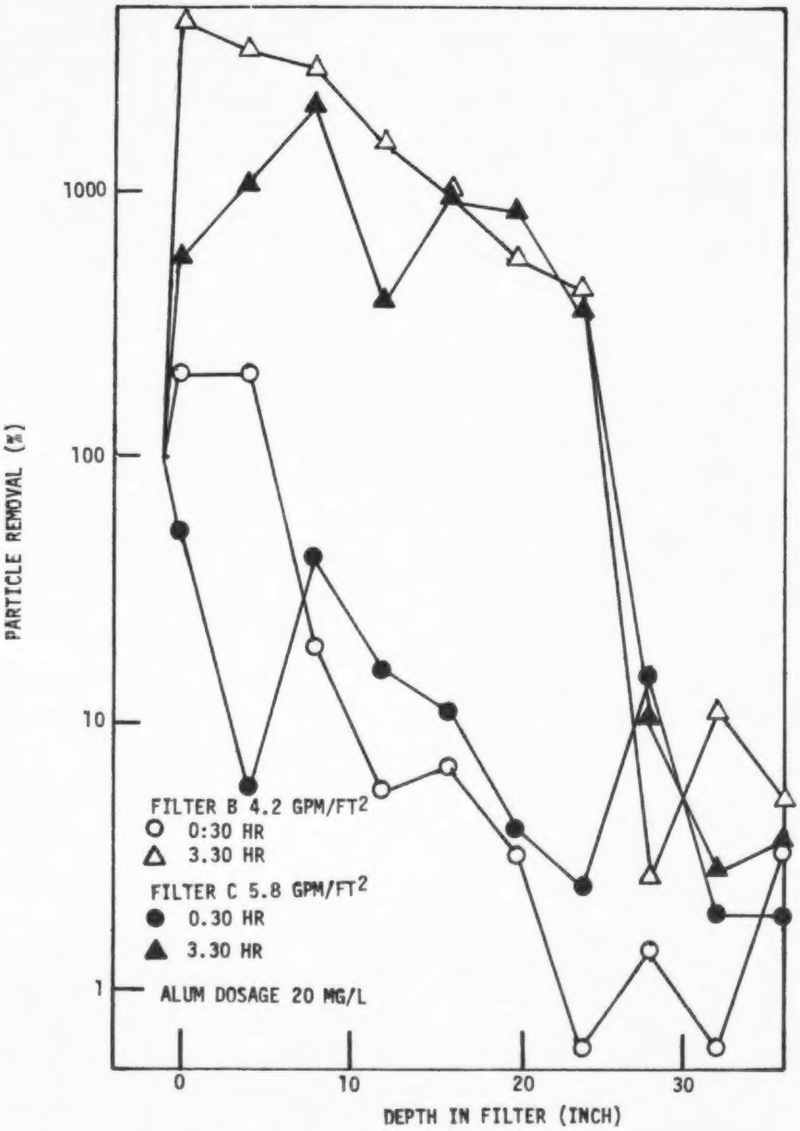


Figure 30. Particle removal at different filter depths.

Direct Filtration at University of Washington with Giardia lamblia Cysts

During the final runs, Filter C was the only filter used for cyst spiking. Furthermore, it was decided for practical reasons to add the cysts as a slug rather than have continuous feeding throughout the run. Continuous feeding would have required extremely large quantities of cysts, because of the high cyst removal efficiency of the filter and the relatively large number of cysts necessary for reliable detection and enumeration in the filter effluent. These large quantities were just not available.

To determine when the peak concentration of cysts would appear in the filter effluent, a series of conductivity tests were made. A salt solution was added to the plant influent at the cyst addition port. The salt solution was added for 30 sec, the same time period that had been selected for the cyst addition. Conductivity was monitored continuously at the final flocculation tank effluent, the overflow from the distribution trough which also functioned as a constant head tank for the filters and the effluent from Filter C. This test was repeated for many different filter loading rates to cover the entire range of normal operation. Knowing the filter loading rate, Figure 31 could be used to determine when sampling started and stopped if the objective was to collect a 20 L filter effluent sample, half of which preceded the peak of the effluent cyst concentration and half of which followed the peak. Figure 32 shows what percentage of all the cysts passing through the filter was captured in the 20 L sample.

G. lamblia cyst stock solutions were prepared by extracting the cysts from stool specimens of giardiasis patients, provided by hospitals and pathology laboratories throughout the State of Washington. The procedure for extraction has been described earlier. The resulting stock solutions ranged in concentration from 1.2×10^5 to 5.0×10^5 cysts/mL, and were stored at 4° C until needed.

Cyst suspensions for the pilot plant runs were prepared immediately before being added to the plant influent. The total cyst concentration selected for the run determined the amount of stock solution used. Distilled water was used as diluent to give a final volume of 180 mL, which was pumped into the plant influent line.

The cyst addition port was located on the plant influent line, ahead of the coagulant feed manifold and opposite the pH adjustment port. A static mixer, Kenics Model 1/2-10-321-5, separated the cyst/pH adjustment feed manifold and the coagulant feed manifold. The static mixer provided good dispersion of the cysts and uniform pH of the raw water before any chemical coagulant was added. The cyst suspension was pumped into the feed line, using a FMI lab pump, calibrated to deliver the 180 mL volume in exactly 30 sec.

Earlier tests aimed at determining cyst losses during the coagulation and flocculation process, had shown some variability when parameters like pH, and coagulant dosage, as well as type of coagulant used were changed. Therefore, during the actual cyst runs both filter influent and effluent

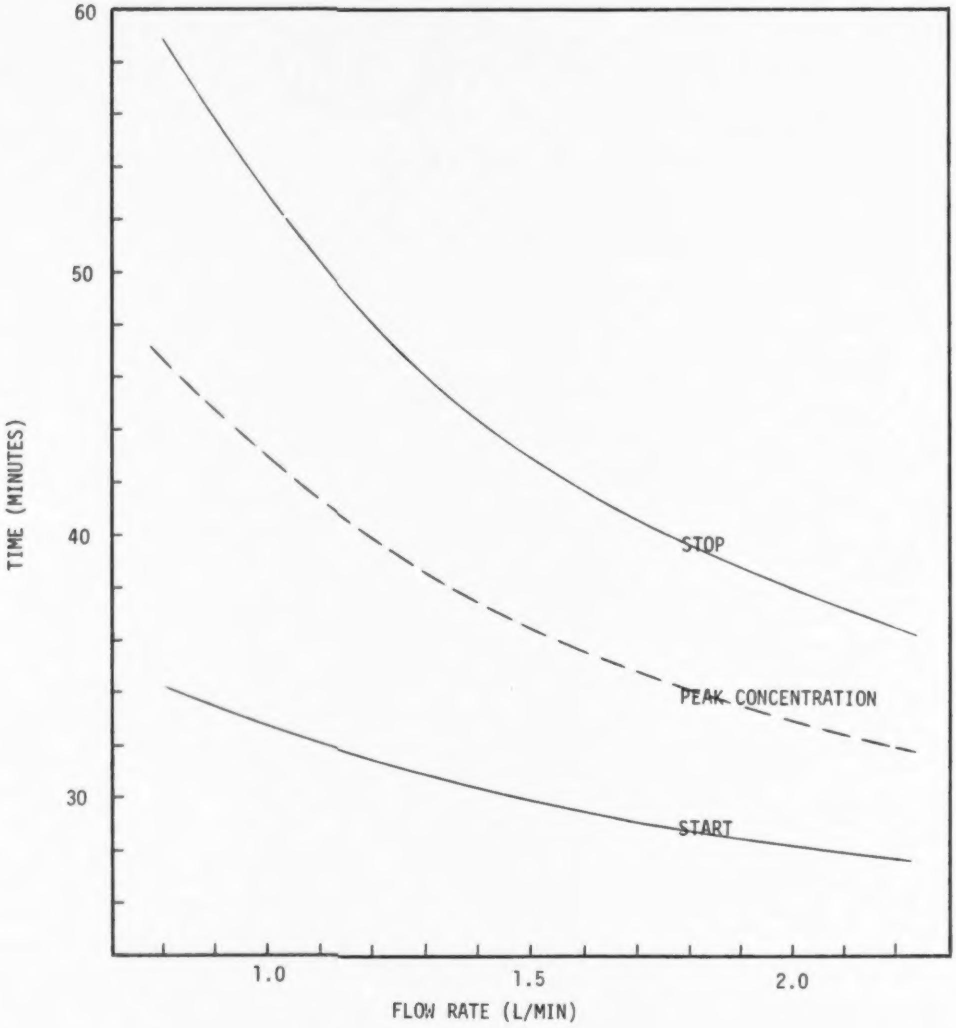


Figure 31. Sampling schedule for 20L filter effluent sample at different filtration rates.

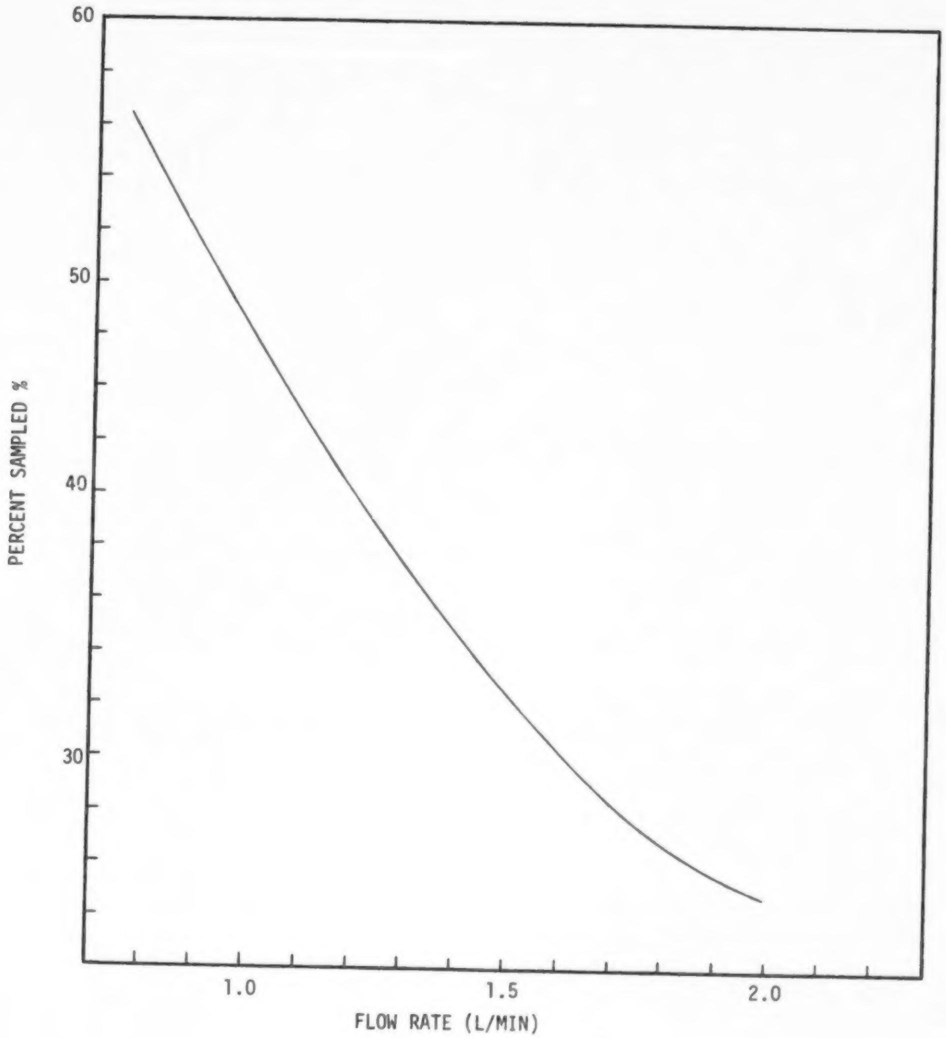


Figure 32. Percentage of total number of filter effluent cysts present in 20L sample collected according to Figure 31.

were sampled to determine the cyst removal efficiency of the filtration process. All samples collected were processed as described earlier, which included membrane filter filtration, centrifugation and microscopic examination.

The cyst dosage during the runs ranged from 2.0×10^6 to 21.5×10^6 cysts. For the majority of runs, however, approximately 20×10^6 cysts were added to the raw water. The total number of organisms actually reaching the filter depended upon two main factors: first, the loss of cysts in the rapid mix and flocculation tanks, due to disintegration and attachment, and second, the filter loading rate. In order to provide a constant reaction time for the coagulation and flocculation processes, the flowrate through the plant was kept constant at 2.3 L/min. For a low filter loading rate, this meant that a proportionately large amount of the cysts reaching the distribution trough would be wasted through the overflow. As the flow rate to the filter increased, so did the cyst load, assuming the dosage to the plant remained relatively constant.

These direct filtration runs were designed to investigate factors such as coagulant dosage and pH, especially its affect on alum coagulation (Table 8). The filter loading rate was kept relatively constant at 9.8 m/hr (4 gpm/ft^2).

As expected, with no coagulant being added to the water, the filter performed poorly with regard to both cyst removal and turbidity reduction. More than half the cysts, 52%, passed through the filter and the effluent turbidity remained relatively high. At optimum conditions, however, cyst removal was consistently high. An alum dosage of 12 mg/L, pH 6.2, and a filter loading rate of 4.9 m/hr (2 gpm/ft^2), would give a 99.73% removal of cysts at the end of the one hour filter ripening period. Later in the run, cyst reduction was 99.94% and the effluent turbidity was constant at 0.02 NTU. The influent turbidity was 1.2 NTU. An increase in the filter loading rate to 9.8 m/hr (4 gpm/ft^2) did not have any adverse effect on the filter's ability to remove cysts. In fact, at the end of the filter ripening period, the cyst reduction was 99.94%, slightly higher than at the lower flowrate. Seven hours into the run it had improved to 99.98%,even though the effluent turbidity was 0.2 NTU, compared to 0.02 NTU at the lower loading rate (Figure 33).

A reduction in the coagulant dosage led, as expected, to an increase in the number of cysts passing through the filter. At a 7 mg/L alum dosage, 99.75% of the cysts were removed one hour into the run, and 99.98% after 16 hrs. The lower alum dosage also resulted in an increase in the filter ripening period to 1.5 hrs and a higher effluent turbidity, 0.03 NTU. A further reduction in the alum dosage to 4 mg/L had a more dramatic effect. The filter ripening period was increased to approximately 2 hrs and only 64.2% of the cysts added to the plant after 2.5 hrs of operation were removed in the filter. The effluent turbidity was 0.5 NTU, but slowly decreasing. The effluent turbidity at 72.5 hrs remained relatively high at 0.4 NTU, whereas the cyst removal had increased to 91.8%.

Table 8. Cyst Removal During Direct Filtration at UW Pilot Plant

Run No.	Alum Coagul. and Dosage Mg/L	pH	Filter Loading Rate m/hr	Filter Infl. Cyst Dosage	Cyst Removal %	Elapsed Time Hrs-Min	Infl. Turbidity NTU	Effl. Turbidity NTU	Turbidity Removal %
72	None	6.5	6.0	$6.6 \cdot 10^5$	48.01	4:30	0.73	0.39	46.58
73	12.0	6.2	6.0	$3.8 \cdot 10^6$	99.733	1:15	1.24	0.03	97.74
			4.3	$4.2 \cdot 10^6$	99.943	26:00	1.19	0.19	98.40
74	12.0	6.2	9.6	$7.3 \cdot 10^6$	99.936	1:00	1:37	0.04	97.23
76	12.0	6.2	9.2	$8.7 \cdot 10^6$	99.979	7:00	1.14	0.02	98.07
77	7.0	6.2	9.6	$9.8 \cdot 10^6$	99.750	1:00	1.94	0.24	87.63
			8.5	$9.4 \cdot 10^6$	99.870	16:00	0.81	0.03	96.30
78	4.0	6.2	10.0	$10.7 \cdot 10^6$	64.23	2:30	1.31	0.52	60.31
			8.5	$8.8 \cdot 10^6$	91.81	72.30	1.35	0.37	72.59
79	12.0	6.8	9.6	$10.3 \cdot 10^6$	95.44	1:00	0.95	0.28	70.53
			8.2	$8.4 \cdot 10^6$	99.41	10:00	1.02	0.04	96.27
80	12.0	5.6	9.6	$10.0 \cdot 10^6$	99.83	1:00	1.73	0.03	98.09
81	12.0	5.6	9.6	$9.8 \cdot 10^6$	99.84	7:00	1.78	0.02	98.93
82	Cat-Floc Tl 5.0	6.4	9.6	$9.8 \cdot 10^6$	95.90	1:00	0.92	0.23	75.00
			9.6	$9.8 \cdot 10^6$	99.911	21:00	0.80	0.27	66.25

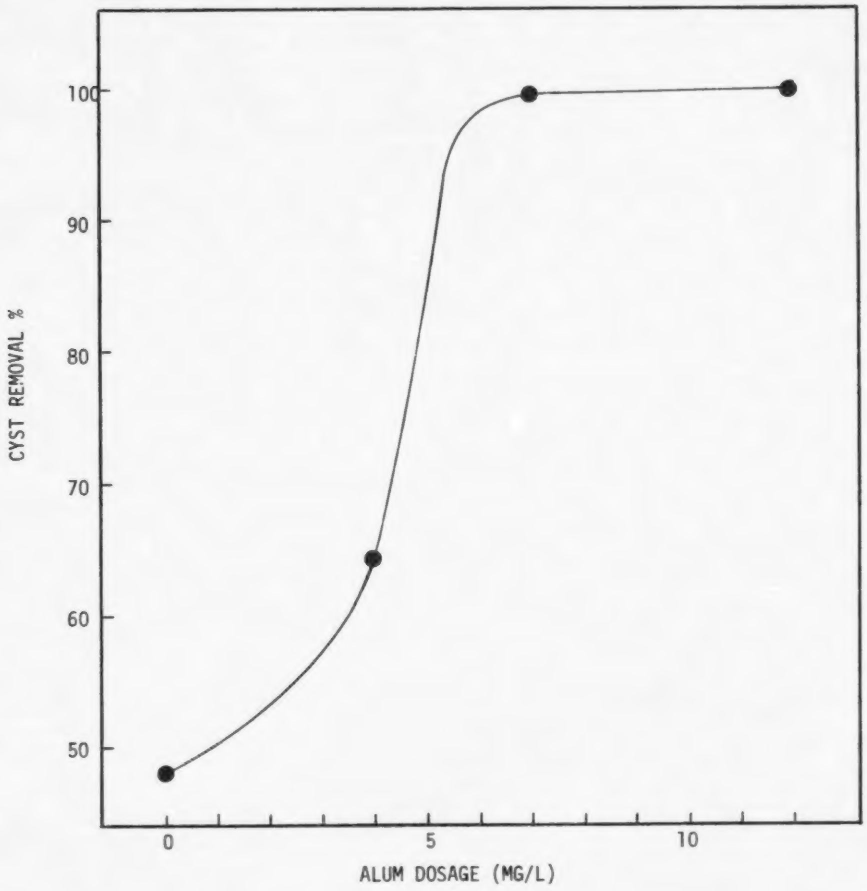


Figure 33. Effect of alum dosage on cyst removal.

During earlier runs with alum as the primary coagulant, the overall performance of the filtration plant was found to be very sensitive to changes in pH. Therefore, some of the cyst runs were designed specifically to investigate the importance of proper pH control on cyst removal. It had been shown that 99.98% of the cysts could be removed during the filtration process with proper pretreatment, using 12 mg/L alum at pH 6.2. Keeping the alum dosage unchanged but lowering the pH to 5.6 did not dramatically affect the cyst removal. After one hour of operation, at the end of the filter ripening period, 99.83% of the cysts were removed by the filter, and 6 hrs later 99.84% were removed. The effluent turbidities were 0.03 NTU and 0.2 NTU, respectively. An increase in pH to 6.8 dropped the cyst removal one hour into the run to 95.44% and the effluent turbidity was 0.3 NTU, showing some fluctuation. After 10 hrs the cyst reduction had improved to 99.41% and the turbidity was at 0.04 NTU (Figure 34).

The only polymer used during the cyst runs was Cat-Floc T1 (Calgon Corp.). A 5 mg/L dosage was determined the optimum and the pH was kept at 6.4, the natural pH of the raw water. The cysts were added to the plant influent after one and 21 hrs of operation with removal efficiency of 95.9% and 99.91%, respectively. Even though good cyst removal was achieved during this run, the effluent turbidity was 0.2 NTU after one hour and increased slightly to 0.3 NTU 21 hrs into the run. These values were considered relatively high in comparison to the excellent filter performance when alum was used as coagulant.

TESTING OF DIATOMACEOUS EARTH FILTER AT THE UNIVERSITY OF WASHINGTON

As expected, the results from the initial runs showed that the cyst-size particle removal by the DE filter was generally better than the reduction in turbidity. Of the several types of filter aid tested, the best performers were the finer grades, especially in the very beginning of the run. Later in the run, however, none specifically outperformed the others. Some typical data are shown in Figures 35, 36 and 37.

The most noticeable difference between these runs was the rate of headloss buildup which was slowest for the coarsest grades. This was also manifested by longer filter runs. The length of the run depended not only on the type of diatomite used, but to a significant degree on the amount of body feed added to the filter. The body feed rate ranged from 10 to 40 mg/L. Though the raw water used for these runs was of high quality, with turbidity normally ranging from 0.5 to 0.9 NTU during this time of the year, body feed rates less than 20 mg/L resulted in relatively short filter runs, usually from 26 to 46 hrs. Similarly, the duration of the filter run decreased if the body feed was increased beyond 20 mg/L.

During one of the runs using Hyflo Super-Cel as filter aid, a low concentration of the nonionic polymer, Magnifloc 985N (American Cyanamid Co., Wayne, NJ) was added to the raw water. The most noticeable effect of the 0.0075 mg/L polymer addition was a significant improvement in the effluent quality in the very beginning of the run. As the run was progressing, the efficiency of the DE filter seemed to be similar to earlier runs where no polymer had been added. The improvement in effluent quality

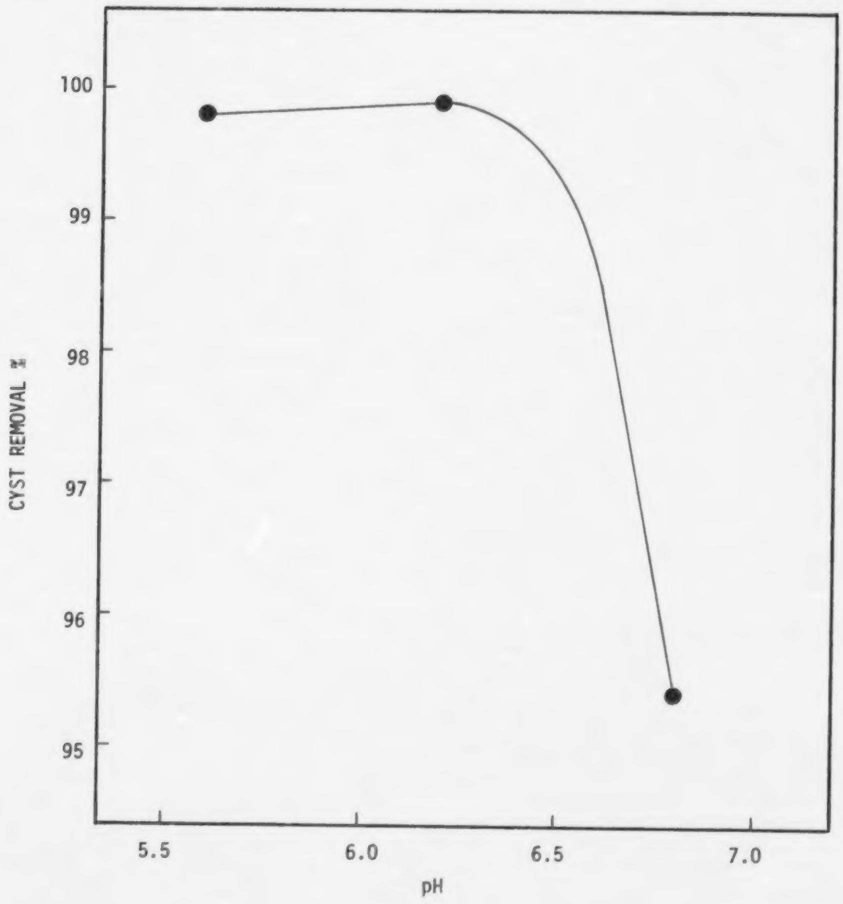


Figure 34. Effect of pH on cyst removal.

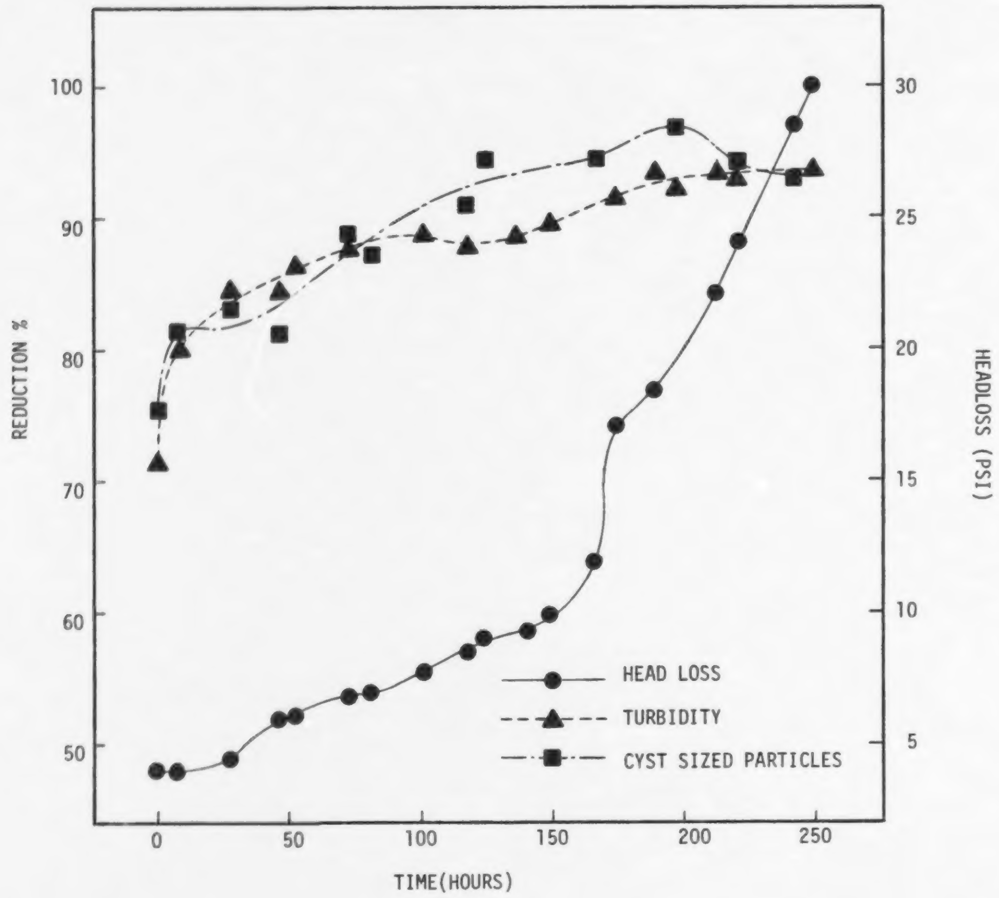


Figure 35. Characteristics of DE filter run with Celite 503 filter aid at 20 mg/L body feed.

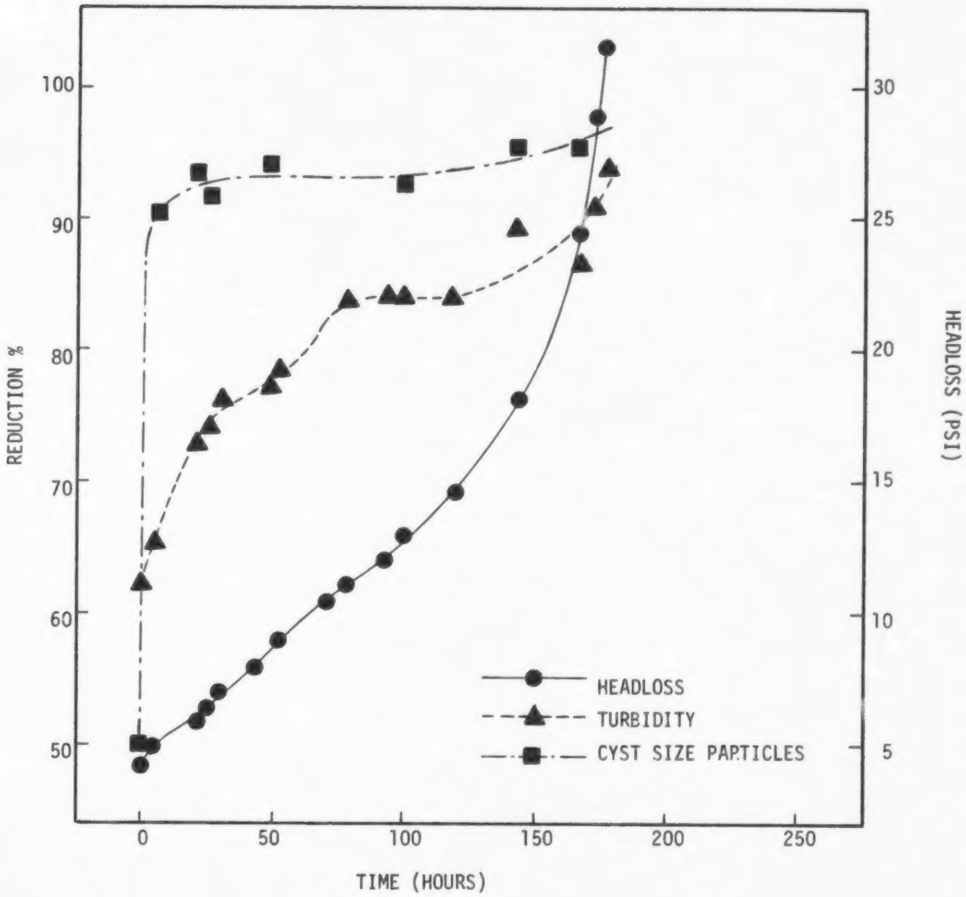


Figure 36. Typical data from a DE filter run using Hyflo Super-Cel as filter aid. Body feed rate, 20 mg/L.

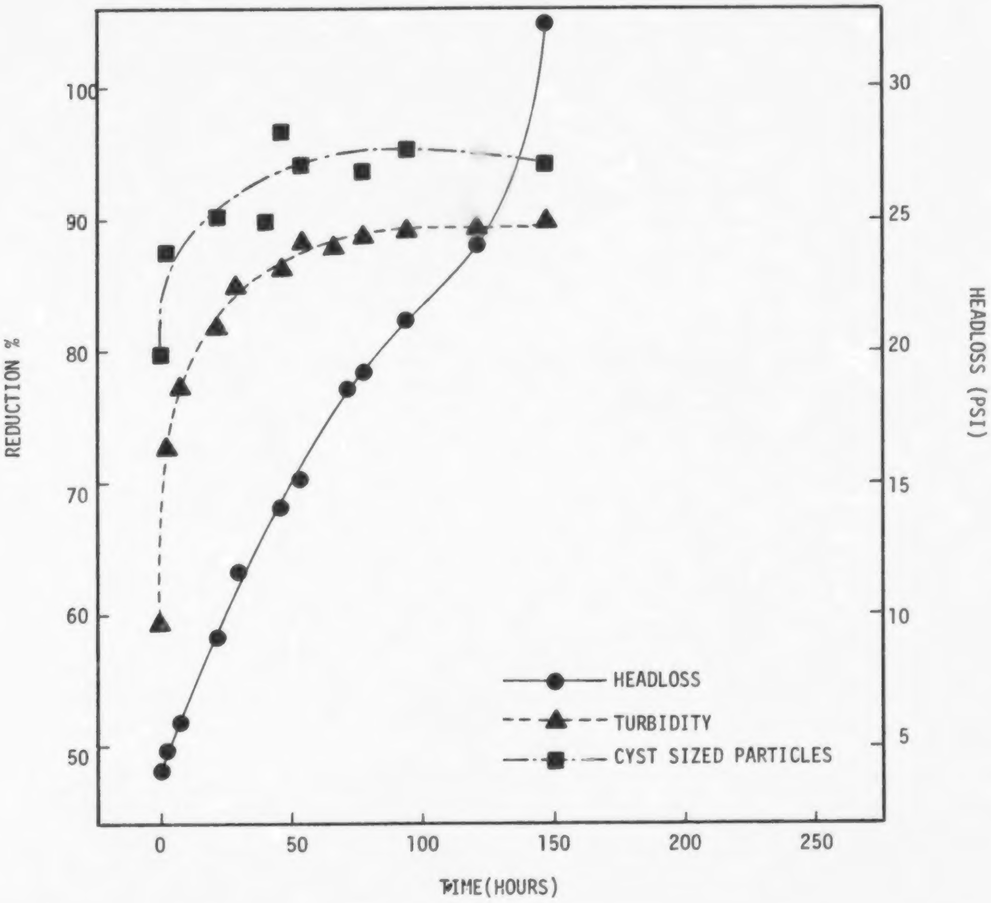


Figure 37. Typical data from a DE filter run using Celite 512 as filter aid. Body feed rate, 20 mg/L.

was paralleled by a more rapid increase in headloss across the filter. When the run was terminated, it was approximately 25% shorter than similar runs where no polymer was added. It was assumed that the single most important factor for the decrease in the duration of the run was the polymer addition.

All effluent samples collected during this series of tests were very low in cysts. In fact, after concentrating the 38 L or 19 L samples to 1 mL with an average recovery of 88.5%, no cysts were detected in 5 of the 12 concentrates. For practical reasons, only aboug 25% of the 1 mL volume was examined. Because of the low counts, the actual cyst removal efficiency of the DE filter could not be determined. Only the boundary values could be determined. However, the data showed that diatomite filtration was effective in removing G. lamblia cysts, even in the very beginning of the filter run when the precoat acted as the only barrier. The only decrease in the filter's ability to trap the cyst particles was recorded when the dosage at the end of the run was increased six times. This decrease in performance was less evident when a polymer was added to the raw water. Generally the polymer addition improved the removal efficiency, but tended to shorten the filter run because of a more rapid rate of headloss buildup, especially towards the end of the run. A better method might be to add polymer only in the beginning. The results are shown in Table 9.

TESTING OF DIRECT FILTRATION IN HOQUIAM AND LEAVENWORTH

Results of EPA Pilot Unit at Hoquiam

The filter runs were conducted at different coagulant dosages, pH values and filtration rates. The main parameters determined were turbidity removal, removal of particles in the 8 to 12 um range, length of filter run and headloss buildup at different depths in the filter.

The major factors influencing treatment efficiency were coagulant dosage, pH and filtration rate. Figure 38 shows the effect of alum dosage at pH 6.7 and 10 m/hr (4.1 gpm/ft^2). The data indicate that the particle removal reached a maximum above a dosage of 10 mg/L, while the turbidity removal was already at its maximum at 8 mg/L alum. Adding 0.04 mg/L of a nonionic polymer, Magnifloc 985N (American Cyanamid Co., Wayne, New Jersey), led to no improvement in the particle or turbidity removal. The rate of headloss buildup, however, increased from 5.3 cm/hr (2.1 in/hr) to 18.3 cm/hr (7.2 in/hr), greatly reducing the length of the filter run. The duration of a filter run could be improved by lowering the coagulant dosage, but the treatment efficiency would suffer as a result.

In general, the rate of headloss buildup was linear with time and the majority of runs were terminated due to turbidity breakthrough before the 205 cm (80 in) to 230 cm (90 in) of available head had been exhausted. The lowest rates of headloss buildup were observed at high pH values and low filtration rates, whereas high rates of headloss buildup were the rule for low pH values and high filter loading rates. The headloss profile at the end of the different filter runs showed a rather uniform distribution throughout the filter, with only a slightly smaller buildup at the top. This indicated that the flocs penetrated the bed sufficiently.

TABLE 9. FILTER RUNS WITH CYSTS USING DE FILTER

		Cyst Addition				
		Number Added				
Run No.	Polymer Added	Slug	Contin. Cysts/l	Elapsed Time Hrs:Min	Removal (R) %	Cyst Size Particle Removal %
63	No	$3.0 \cdot 10^6$		0:05	$99.35 < R < 99.78$	98.4
		$3.0 \cdot 10^6$		0:20	$99.65 < R$	95.1
		$3.0 \cdot 10^6$		2:00	$99.65 < R$	97.9
			$1.5 \cdot 10^5$	2:30	$99.61 < R < 99.96$	98.8
			$9.0 \cdot 10^5$	3:00	$99.03 < R < 99.10$	92.3
64	Yes	$3.0 \cdot 10^6$		0:05	$99.61 < R < 99.96$	98.1
		$3.0 \cdot 10^6$		0:20	$99.65 < R$	98.2
		$3.0 \cdot 10^6$		2:00	$99.65 < R$	98.2
			$1.5 \cdot 10^5$	2:30	$99.65 < R$	97.9
			$9.0 \cdot 10^5$	3:00	$99.48 < R < 99.56$	96.3
65	No		$4.5 \cdot 10^5$	3:00	$99.83 < R < 99.94$	87.1
66	Yes		$4.5 \cdot 10^5$	3:00	$99.87 < R < 99.99$	95.0

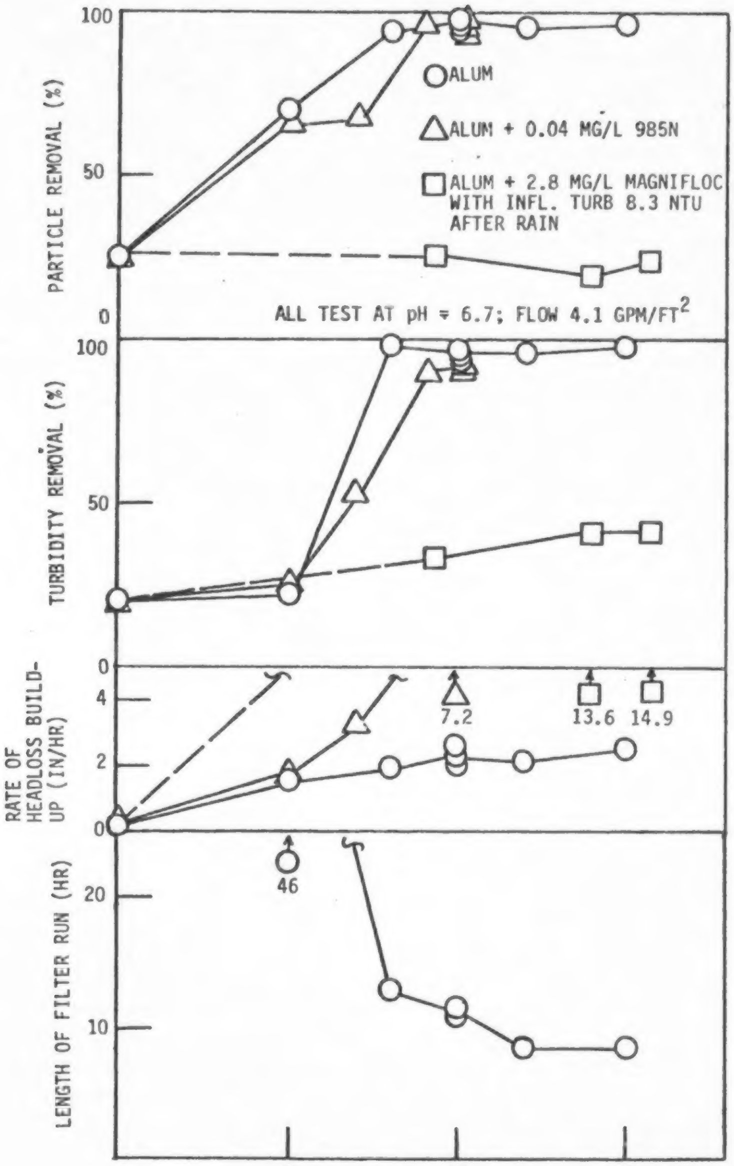


Figure 38. Effect of alum dosage on particle and turbidity removal during field work at Hoquiam.

A series of runs were made with polymer as primary coagulant or coagulant aid in combination with alum. The data indicated that 3.4 mg/L Catfloc T (Calgon Corp., Pittsburgh, PA) or 2.1 mg/L Magnifloc 573C (American Cyanamid Co., Wayne, NJ) were required to obtain a larger than 90% particle and turbidity removal. A 5 mg/L alum dosage with polymer coagulant aid showed no major improvement in particle and turbidity removal as compared to the same polymer dosage by itself. When the alum dosage was increased to 7.1 mg/L the filter's effectiveness improved and better than 90% removal of turbidity and cyst-sized particles was observed at the 7.1 mg/L alum dosage in combination with either 1.7 mg/L Catfloc T or 2.0 mg/L Magnifloc 573C. Without changing the polymer dosage, no significant improvement was evident when the alum dosage was increased beyond 8.1 mg/L. The addition of polymer generally tended to decrease the rate of headloss buildup, possibly by forming flocs that penetrated deeper into the filter bed.

Another important parameter for controlling particle and turbidity removal, particularly when alum was used as coagulant, was pH. The data in Figure 39 show that at Hoquiam the highest removals were obtained at a pH value of 6.7, which remained optimum throughout the study period, with only one exception. High removals were still observed in the 6.4 to 7.0 pH range, but lower removals were noted outside this range. Lowering the pH to 6.0 resulted in particle and turbidity removals below 90% and caused a high rate of headloss buildup. Similarly, an increase in the pH to 7.4 resulted in a major decrease in turbidity removal, but a lesser decrease in the removal of particles. In the present study, the pH was manipulated by the addition of hydrochloric acid (HCl) or soda ash (Na_2CO_3). The latter could be dosed more accurately than the lime previously used. To maintain optimum pH during alum addition, soda ash was always required to counteract the pH decrease caused by the alum. The addition of alum decreased the pH as it decreased the alkalinity as given by:

$$Al_2(SO_4)_3 \cdot 14.3\ H_2O + 3\ Na_2CO_3 + {}_3H_2O \rightarrow {}_2Al(OH)_3 \downarrow + {}_3Na_2SO_4 + 14.3\ H_2O + 3\ CO_2$$

During the pilot plant study, the addition of 10 mg/L alum typically resulted in a pH decrease of 0.27 pH units. Addition of 10 mg/L soda ash generally increased the pH 0.46 units.

The effect of sudden changes in pH is demonstrated in Figure 40. The data were obtained from two runs with an alum dosage of 10 mg/L and a 10 m/hr (4.1 gpm/ft^2) filtration rate. During the first run the pH changed from 6.8 to 7.3, resulting in a decrease in particle removal and the rate of headloss buildup. An increase in effluent turbidity was also noticed, approaching the influent turbidity of 1.7 NTU. The increase to pH 6.9 only changed the rate of headloss buildup. However, the change from pH 6.9 to 7.3 had the largest impact as evidenced by an increase in effluent turbidity of 0.5 NTU. In addition, particle removal and rate of headloss buildup decreased. When the pH was brought back to 6.8 the effluent turbidity responded immediately and returned to its initial value.

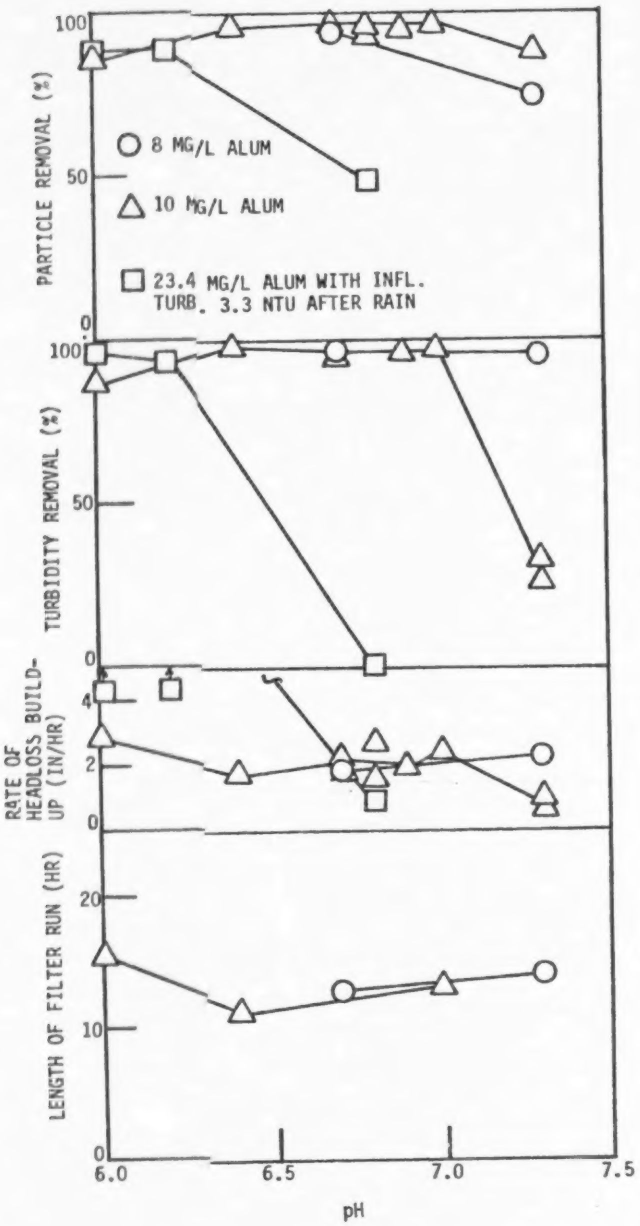


Figure 39. Effect of pH on particle and turbidity removal during field work at Hoquiam.

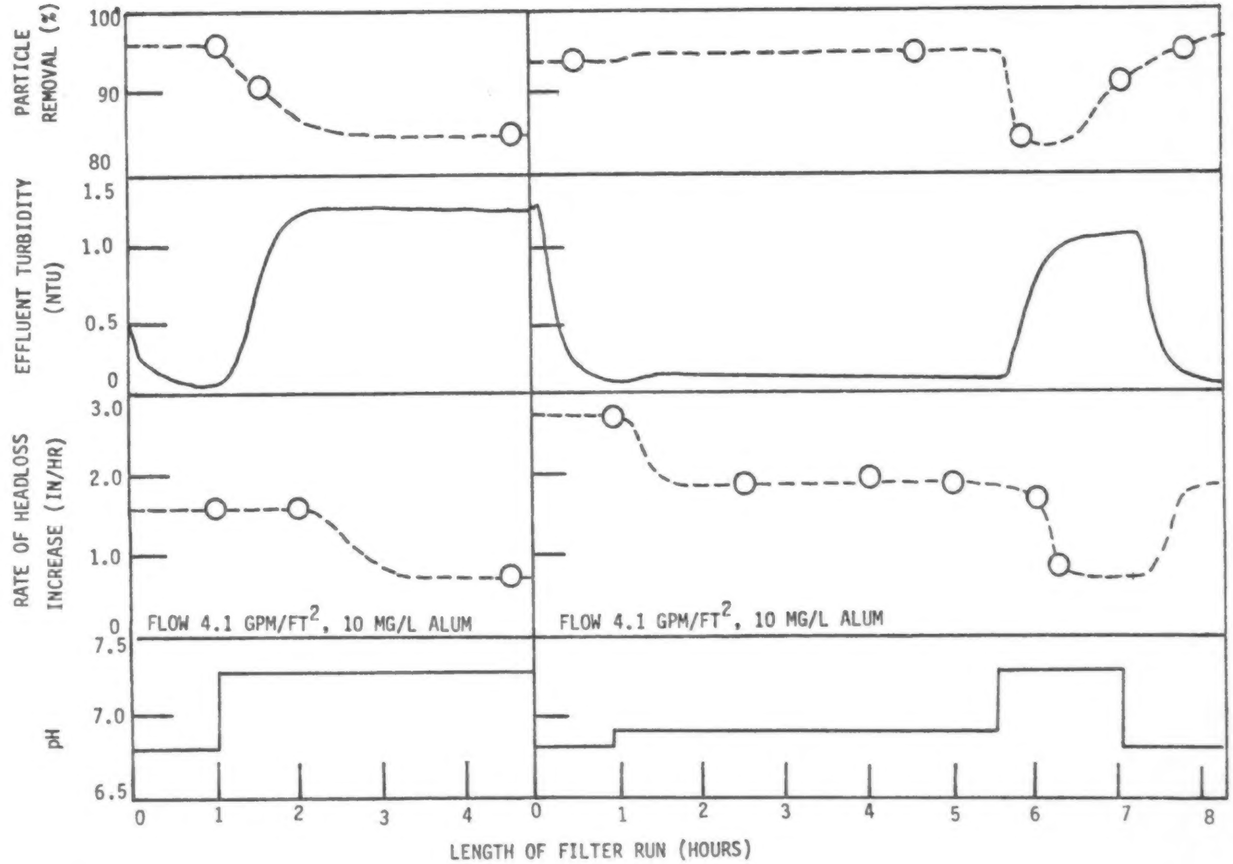


Figure 40. Effect of pH changes during Run No. 9 at Hoquiam.

Increasing the filtration rate from 10 m/hr (4.1 gpm/ft^2) to 15 m/hr (6.1 gpm/ft^2) was generally detrimental to the overall performance of the filter (Figure 41). At an alum dosage of 15 mg/L and a pH of 6.7, the higher filtration rate resulted in a gradual decrease in turbidity and particle removals. Further, the higher filtration rate resulted in a more rapid buildup of headloss and a significant shortening of the filter run due to an early turbidity breakthrough (Figure 42). Because of the short filter run, less than half as much high quality water was produced at this filtration rate as would normally be expected at 10 m/hr (4.1 gpm/ft^2) with the same raw water quality.

A very low turbidity and particle removal (49 and 48%) was experienced on September 2, following a rainstorm which increased the influent turbidity from 1.2 to 8.3 NTU. A more than doubling of the alum dosage to 23.4 mg/L, in combination with a lowering of the pH to 6.0 was required to bring the effluent quality to within normal operating values. In addition to an increase in turbidity during the heavy rain, the pH of the raw water decreased from 7.3 to 6.8. These results indicate that optimum process conditions can change rapidly, within a few hours, as changes occur in the quality of the raw water.

A relationship was established between effluent turbidity and particle removal (Figure 43). An effluent turbidity below 0.05 NTU corresponded with a median (50% of the values) particle removal of 95.1%, while an effluent turbidity between 0.05 and 0.1 NTU was associated with a 94.3% particle removal. A surprisingly large number of samples (33%) with an effluent turbidity below 0.05 NTU had particle removals below 90%. This was observed especially in the beginning of the run directly after the filter ripening or during the running phase when the influent particle concentration declined temporarily. The polymer plus alum and polymer runs did not produce effluent turbidities below 0.1 NTU, but high median particle removals of 95.3 and 92.6% respectively, were noted for effluent turbidities between 0.10 and 0.20 NTU. These results are further summarized in Figure 44 which shows a relationship between median particle removal and effluent turbidity range. Greater than 90% median particle removal was observed for effluent qualities below 0.2 NTU but not for values above it.

The study also evaluated the removal of actual Giardia lamblia cysts by the pilot unit. Cysts recovered from human stool specimens were added to the raw water, ahead of any chemical addition, during an 8 min spike. The cysts were recovered from the influent and effluent using a membrane filtration technique. Of the 1.67 x 10^6 cysts added to the raw water, 1.06 x 10^3 remained in the water just before entering the filter according to the membrane filtration technique. The filter effluent contained a total of 2.6 x 10^2 cysts, representing at least an 81% cyst removal. This corresponded with a 99% removal of particles in the 8 to 12 um range as determined by the particle counter and a 94% turbidity removal.

Full Scale Plant at Hoquiam

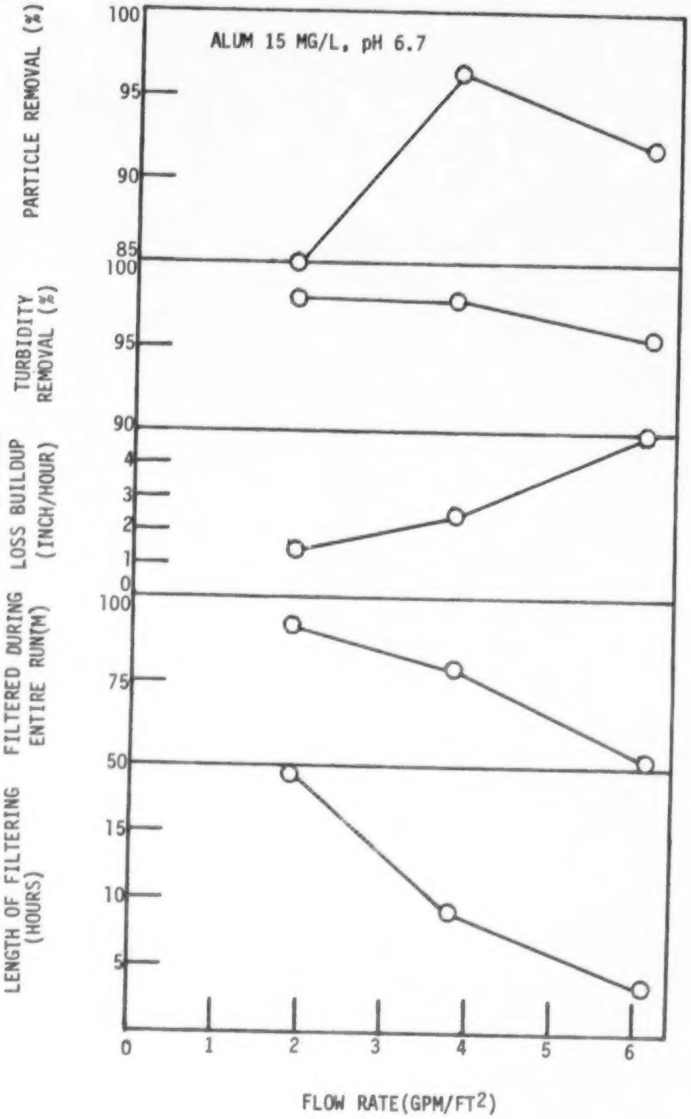


Figure 41. Effect of filtration rate on particle and turbidity at Hoquiam.

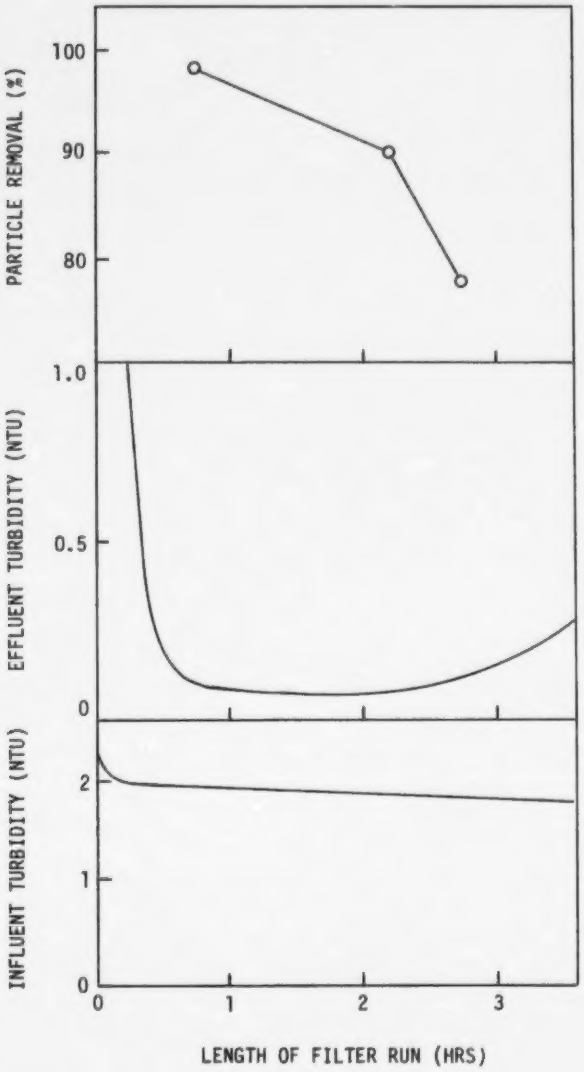


Figure 42. Effect of high filtration rate on filter performance at Hoquiam. Alum dosage 15 mg/L, pH 6.7 and filter loading 15 m/hr (6.1 gpm/ft^2).

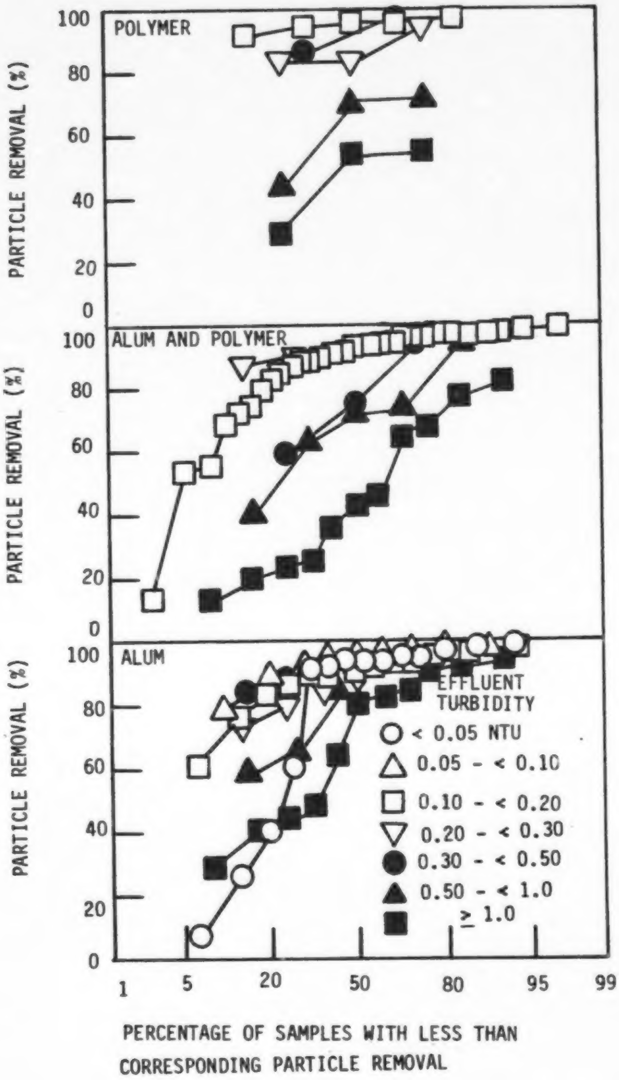


Figure 43. Relationship between effluent turbidity and particle removal at Hoquiam.

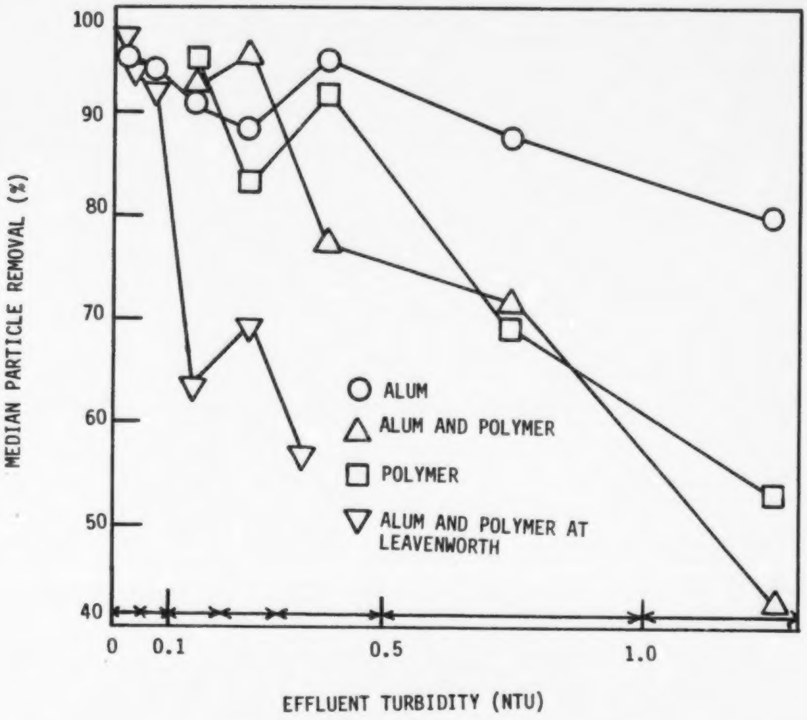


Figure 44. Relationship between effluent turbidity and median particle removal

The City of Hoquaim water treatment plant used both alum and polymer for pretreatment. Normal practice was to add the polymer, at 1 mg/L or less, as primary coagulant and filter aid during periods of low turbidity. When the raw water turbidity exceeded 1.5 NTU, alum was used as primary coagulant and the polymer as coagulant aid and filter aid. The alum dosage could be as high as 30 mg/L depending upon the raw water quality. Alum was also used to precoat the filters following backwash. The pH was controlled by the addition of soda ash.

The chemicals, including chlorine gas for prechlorination, were added to the raw water line about 30 meters (100 ft) upstream of the flocculator. Powdered activated carbon was added just ahead of the flocculator for removal of color. The flow ranged from 8400 m^3 (1.8 mgd) to 12,100 m^3/day (3.2 mgd). At average flow, the retention time in the flocculator was 9 min, and 67 min in the sedimentation basin, corresponding to an overflow rate of 61.6 m/day (1467 gpd/ft^2). The filter loading rate was 9.0 m/hr (3.7 gpm/ft^2).

The plant results in Figure 45 show that the percent turbidity removal increased with increasing influent turbidity, whereas the effluent turbidity was not greatly affected by the higher influent values. A comparison of turbidity removals with and without alum addition indicates that no major benefit resulted from its use. This is further illustrated in Figure 46 where an alum dosage of 20 mg/L resulted in turbidity removals ranging from 50 to 98%.

The high removal variability was primarily due to fluctuations in pH during coagulation and flocculation. The pH ranged from 6.6 to 7.4 and the lower removals were observed at the higher pH values. The apparent inability of alum to affect the overall performance of the plant at other dosages was again related to operating at high pH values.

Data obtained by the EPA pilot plant treating the exact same water, had indicated the pH optimum to be at 6.7 pH units. At this pH, an alum dosage of 10 mg/L and a 10 m/hr (4.1 gpm/ft^2) filtration rate, the pilot plant reduced the turbidity to 0.03 NTU, a 98.3% reduction. In fact, the lowest daily average effluent turbidity at the full scale plant during the study period, 0.25 NTU, occurred at a process pH of 6.7. The alum dosage was 22 mg/L and the filter loading rate 7.2 m/hr (3.0 gpm/ft^2). The raw water turbidity that day was 2.0 NTU, thus yielding an 87.5% reduction. It was felt that the alum dosage could have been reduced without adversely affecting plant performance. Possible benefits from an efficiency standpoint, would be lower effluent turbidity and longer filter runs.

Some operational changes at the plant were considered as a result of the pilot plant work. One of them, a closer monitoring of the raw water pH as it entered the flocculator, was well underway toward the end of the study. This included reducing the amount of soda ash added to the raw water. Instead, additional soda ash was added to the clearwell to increase the pH before distribution as a corrosion control measure.

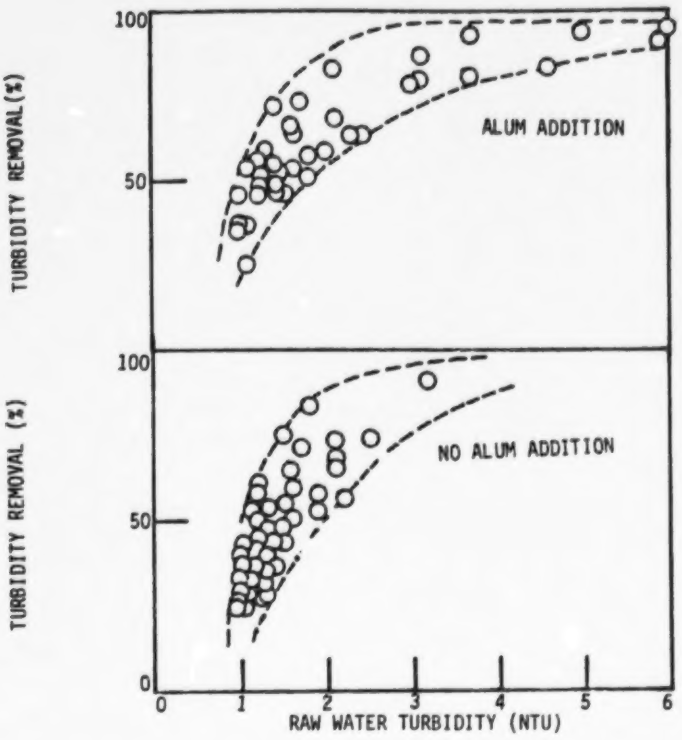


Figure 45. Turbidity removal at Hoquiam Water Treatment Plant.

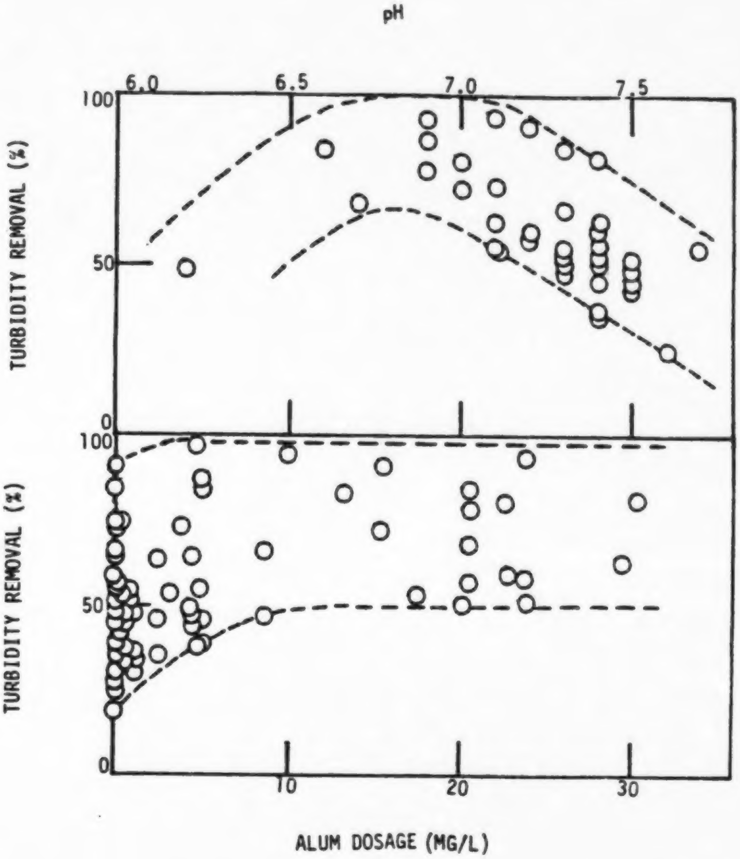


Figure 46. Effect of alum dosage and pH on turbidity removal at Hoquiam Water Treatment Plant.

EPA Pilot Plant at Leavenworth

Water to the pilot plant was supplied from a fire hydrant located on the raw water line directly adjacent to the City of Leavenworth Water Treatment Plant. The raw water turbidities were very low during the fall, generally around 0.3 NTU, with a range of 0.22 to 0.85 NTU. During September and October the water temperature averaged 8.5° C, alkalinity 24.0 mg/L and pH 6.8.

Most of the testing was conducted at a filtration rate of 10.7 m/hr (4.4 gpm/ft^2) which corresponded to the maximum filter loading rate at the full scale plant. The optimum alum dosage during this time period was 15 mg/L, resulting in a 90% reduction in turbidity. The corresponding cyst-sized particle removal was 96%, with the maximum 98% occurring at an alum dosage of 13 mg/L (Figure 47). The pH optimum was 6.7. At pH 6.4 and 7.1 both turbidity and particle removals were reduced (Figure 48).

The influent turbidity, as indicated in Figure 48, showed some variability. However, the highest recorded value, 0.85 NTU, was an isolated peak associated with a heavy rainstorm. The more moderate fluctuations did not seem to have much impact on the effluent quality. For the most part, the effluent turbidity would vary from 0.02 to 0.03 NTU when the plant was operated at or near optimum conditions. Since this threshold value was below the limit of sensitivity claimed by the manufacturer of the flow-through turbidimeters used, it was verified by grab samples on a bench model.

During the month of November the average water temperature dropped to 3° C, the alkalinity decreased to 12.5 mg/L and the pH to 6.4. These changes had a noticeable impact on the effluent quality. The turbidity removal decreased from 90 to 50%, and only 48% of the particles were retained by the filter. To improve upon the plant's performance, a new evaluation of the optimum operating conditions was made. It indicated that the optimum alum dosage had been reduced from 15 to 7 mg/L and the pH optimum increased from 6.7 to 7.0. However, the performance was poor compared to earlier runs, and the effluent turbidity did not reach a stable value following filter ripening, but decreased rather slowly throughout the run. As a result the turbidity removal was at times lower than the 50% experienced when no coagulant was used, and was never better than 61%. It was suspected that because of the low water temperature, the 8 min retention time in the flocculator was inadequate for proper floc formation. Hence, the necessary pretreatment was not achieved before filtration. To further investigate this theory, raw and finished water was analyzed for aluminum. It was not surprising to find that at times as much as 70% of the alum coagulant added was passing through the filter. When the Magnifloc 985N was used as filter aid, an increase in particle removal up to a dosage of 0.026 mg/L was noted. A lesser improvement was observed in turbidity removal. The addition of Calgon L-650E as filter aid lowered turbidity and particle removal.

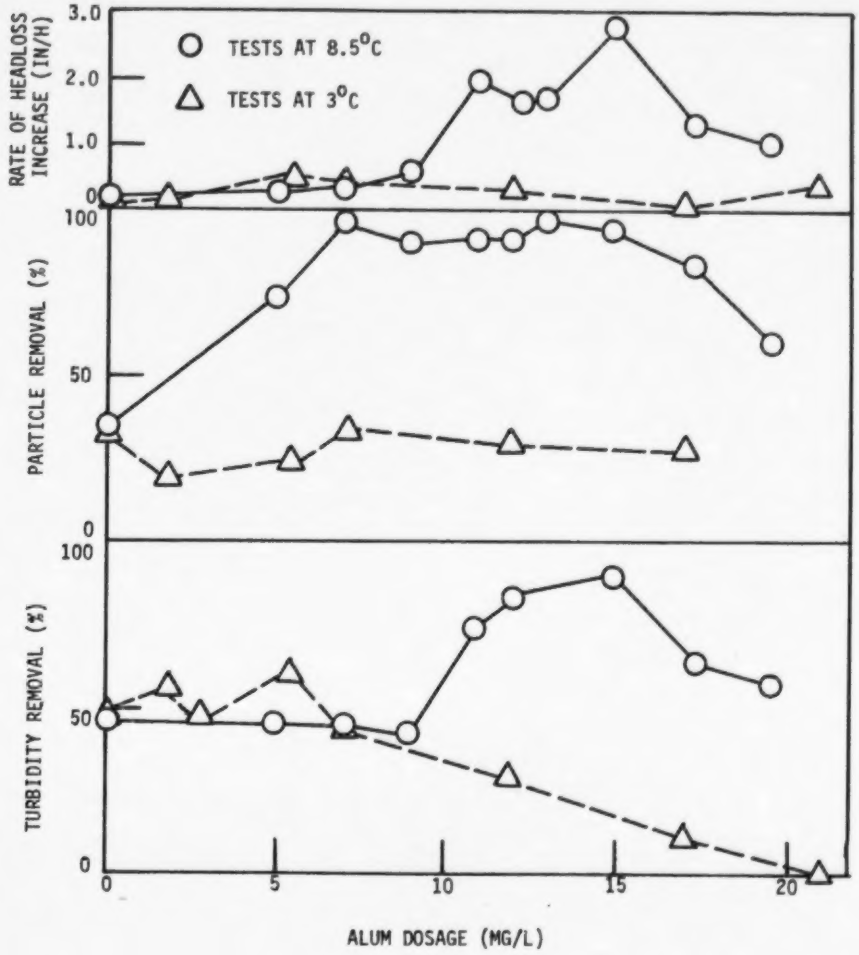


Figure 47. Effect of alum dosage and turbidity removal at different temperatures during field work at Leavenworth.

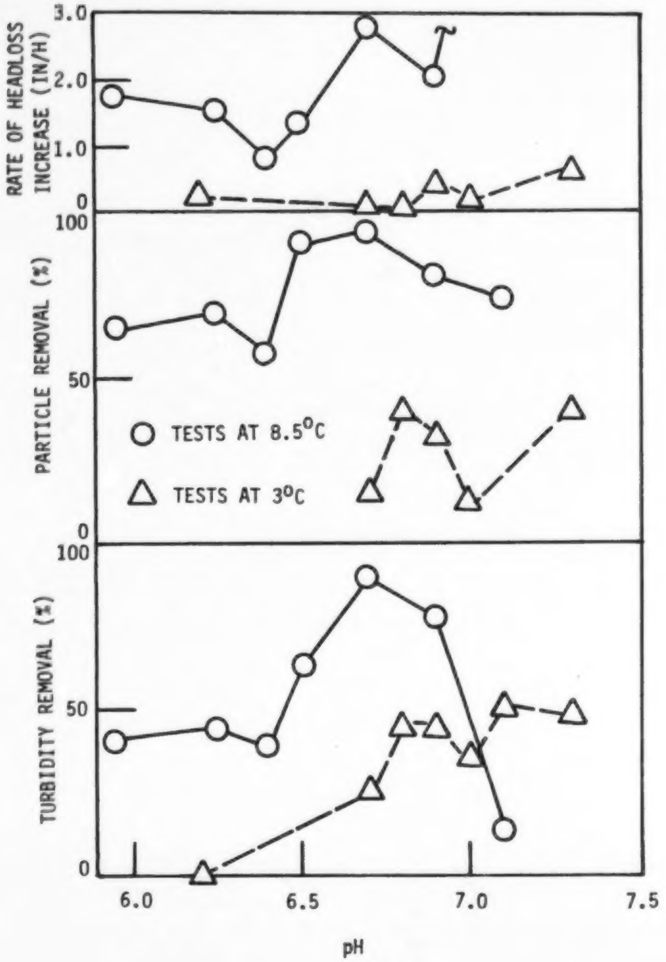


Table 48. Effect of pH on particle and turbidity removal at different temperatures during field work at Leavenworth.

Polymers as primary coagulant at 2°C showed removals comparable to that experienced with alum at this low temperature (Figure 49). Maximum turbidity removal of 59% was realized at a dosage of 0.2 to 0.4 mg/L, compared to 43% removal with no coagulant. The particle removal decreased at low temperatures from 45 to 12% when 0.2 to 0.4 mg/L of polymer was added. To optimize particle removal under these conditions, a polymer dosage of 3.5 mg/L was required. However, the high polymer dosage decreased the turbidity removal to 35%, apparently a result of the colloidal restabilization by the polymer.

A greater cyst-sized particle removal was noticed when lower effluent turbidities were reached. A frequency distribution plot (Figure 50) of the alum runs at 8° C and 3° C show that a median particle removal of more than 90% was realized at an effluent turbidity of 0.1 NTU. Median particle removals of 64 and 68% were realized at an effluent turbidity of 0.1 to 0.2 NTU and 0.2 to 0.3 NTU, respectively, while lower particle removals were associated with higher effluent turbidity values. The results from the runs with polymer (Cat Floc T) as primary coagulant showed a trend opposite that of the alum data, as the lowest particle removals were observed at the lowest effluent turbidity values. An effluent turbidity of 0.1 to 0.2 NTU gave a 53% median removal. At effluent turbidities between 0.1 and 0.3 NTU, better particle removals were obtained with alum than polymer, but the opposite was true above 0.3 NTU.

To determine the ability of the pilot plant to remove cysts, 1.25 x 10^6 cysts were added to the raw water over a 320 min period, and the filter influent and effluent sampled and analyzed for cysts. Prior to the cyst addition, a salt solution had been added to the influent water and traced through the plant to determine suitable sampling times. The plant was operated at 10.7 m/hr (4.4 gpm/ft^2) filtration rate with 1.2 mg/L Cat Floc T as the coagulant. The raw water turbidity was 0.33 NTU and the temperature 1° C. During the cyst addition the effluent turbidity was 0.19 NTU, a 42.4% reduction. The three filter influent samples recovered a calculated 867 cysts while 242 cysts were recovered from the effluent corresponding to a 72.1% removal. The particle removal was 53.3%.

Full Scale Plant at Leavenworth

The full scale plant was operated at polymer dosages ranging from 0.2 to 0.6 mg/L of Cat Floc T. No improvement in turbidity removal was noted at higher dosages (Figure 51). In addition, 0.06 mg/L L-650E was added to the inlet flume, ahead of the filters, as a filter aid. At an influent turbidity of 0.30 NTU which was quite common during dry weather, the effluent turbidity was 0.13 NTU or a 57% removal. Higher removals were only associated with higher raw water turbidities. A 0.21 mg/L dosage of Cat Floc T at an influent turbidity of 1.0 NTU resulted in an 84% removal.

During most of the study period, some of the solenoid valves controlling filter operation did not function properly. The result was a loss of vacuum. This sometimes occurred during the night when the plant was

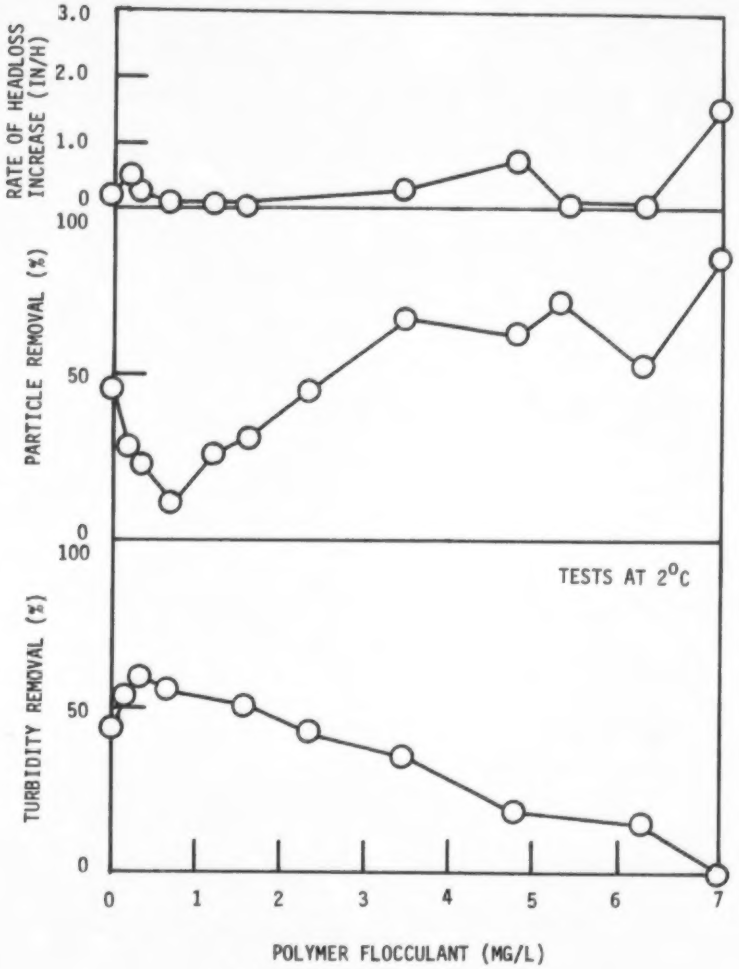


Figure 49. Effect of Cat Floc T polymer dosage on particle and turbidity removal and rate of headloss buildup at Leavenworth.

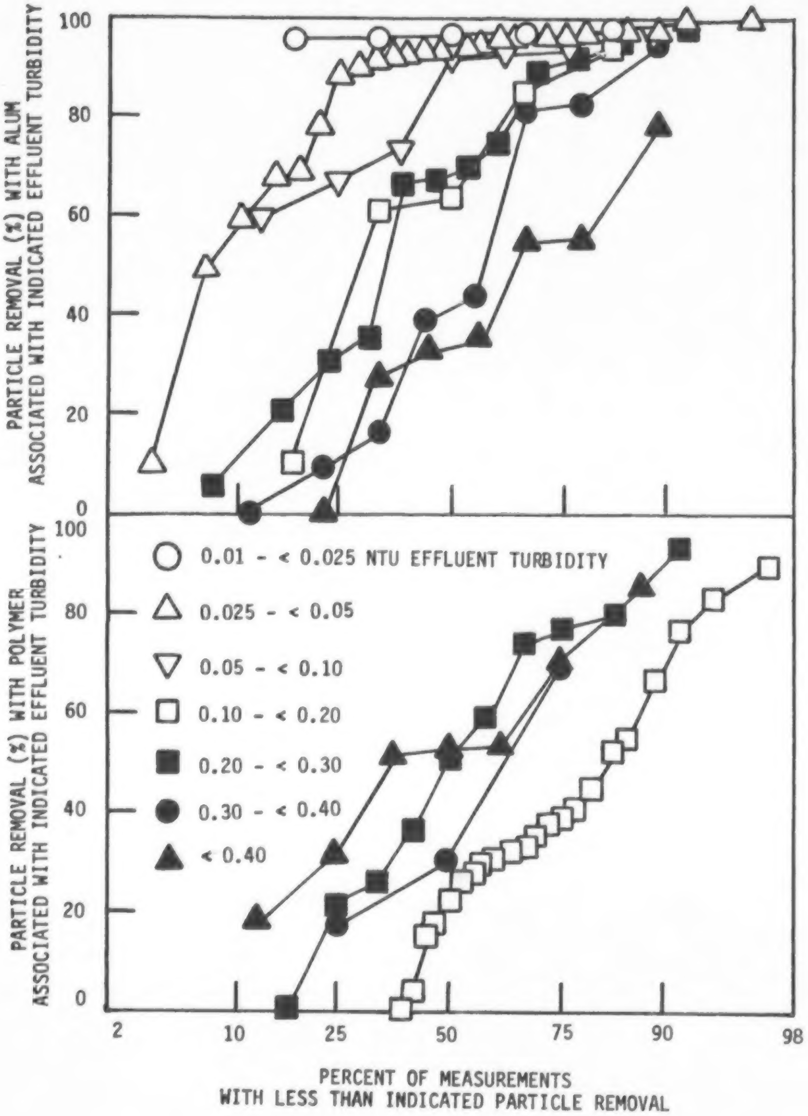


Figure 50. Frequency distribution of particle removal at different effluent turbidities during alum and polymer treatment at Leavenworth.

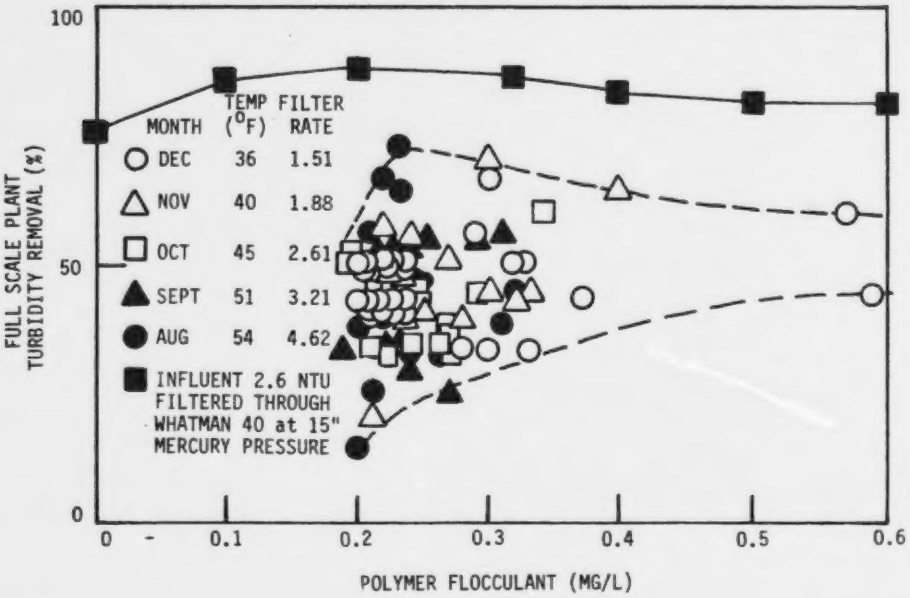


Figure 51. Effect of polymer dosage on turbidity removal at Leavenworth Water Treatment Plant

unattended. Without vacuum none of the siphons could be initiated. As a result, if a filter reached terminal headloss, the inlet siphon would be broken. With no vacuum it could not be backwashed. This could, in fact, shut down the entire plant until the vacuum was reestablished.

Summary of Field Activities

During the nearly seven months of field operation, a total of 49 runs were made. Of these, 30 were performed at Hoquiam between May 7 and September 5, 1980.

Although the City of Hoquiam Water Treatment Plant was a conventional plant whereas the pilot plant was operated as a direct filtration plant, much of the information obtained by this study could be and was used to evaluate the full scale plant operation. Having identified the relative importance of the key unit process variables, a few operational changes relating to chemical addition were made. The benefit from these changes was improved pH control during alum coagulation and flocculation and better utilization of the nonionic polymer when used as a filter aid.

From September 17 through November 29, 1980, the pilot plant was operated at Leavenworth. A total of 19 runs were made. Cold weather during the last two weeks of operation caused the water temperature to decrease significantly, fluctuating between 2 and 3°C. The result was poor floc formation, particularly when alum was used as the coagulant.

The city's treatment plant did at times experience operational problems due to equipment malfunctioning, however, these were later corrected and new equipment was installed to improve the rapid mix process.

REFERENCES

Boeck WC. On the longevity of human intestinal protozoan cysts. Amer. Jour Hygiene 1:527-540, 1921.

Center for Disease Control. Giardiasis, Vail Colorado. Morbidity, Mortality Weekly Report 27:155, 1979.

Center for Disease Control. Intestinal parasite surveillance. Annual Summary, Atlanta, 1979.

Chandler AC and Read CP. Introduction to parasitology. John Wiley and Sons, New York, 1961, p. 10.

Davis RB and Hibler CP. Animal reservoir and cross species transmission of Giardia. In: Waterborne transmission of giardiasis. USEPA, Cincinnati, EPA 600/19-79-001, 1979.

Dobell CA. The discovery of intestinal protozoa in man. Proc Royal Soc Med 13:1-15, 1920.

Fantham HB and Porter A. The pathogenicity of Giardia (lamblia) intestinalis to men and experimental animals, Brit Med Jour 2:139-141, 1916.

Frost F, Plan B, Liechty B. Giardia prevalence in commercially trapped mammals. Jour Environm Health 42:245-249, 1980.

Goodbar JP. Join symptoms in giardiasis. Lancet 1:1010-1011, 1977.

Kirner J, Littler JD, Angelo LA. A waterborne outbreak of giardiasis in Camas. Jour Amer Water Works Assoc 70:35-40, 1978.

Kafoid CA and Christiansen EH. On the life history of Giardia. Proc Nat Acad Sci 1:547, 1915.

Konenenko YM. Erythema multiform exudatinum in a child with lamblia cholycystitis. Pediatr Akush Genekol 2:30-31, 1976.

Levine ND. Giardia lamblia: classification, structure, identification. In: Waterborne transmission of Giardiasis, USEPA 600/19-79-001, Cincinnati, 1979.

Lippy EC. Tracing a giardiasis outbreak at Berlin, New Hampshire. Amer Water Works Assoc 512-520, 1978.

Lopez CE, Dykes AC, Juranek DD, Sinclair SP, Conn JM, Christie RW, Lippy EC, Schultz MG, Mires MH. Waterborne giardiasis: a community wide outbreak of disease and a high rate of asymptomatic infection. Amer Jour Epid 112:495-506, 1980.

Moore GT, Cross WM, McGuire D, Mollahan CS, Gleason NN, Healy GR and Newton LH. Epidemic giardiasis at a ski resort. New England J Med 281:402-407, 1969.

Rebhun M, and Argaman Y. Evaluation and Hydraulic Efficiency of Sedimentation Basins. Jour SED, ASCE 91:37, 1965.

Rendtorff RC and Holt CJ. The experimental transmission of human intestinal protozoan parasites IV: Attempts to transmit Endomoeba coli and Giardia lamblia cysts by water. Amer Jour of Hygiene 60:327-328, 1954.

Shaw PK, Brodsky RE, Lyman DO, Wood BT, Hibler CP, Healy GR, McCleod KI, Stahl W and Schultz MG. A community-wide outbreak of giardiasis with evidence of transmission by a municipal water supply. Ann Intern Med 87:426-432, 1975.

Sheffield HG and Bjorvatn B. Ultrastructure of the cyst of Giardia lamblia. Amer Jour Trop Med Hygiene 26:23-30, 1977.

Schultz MG. Giardiasis. Jour Amer Med Assoc 223:1383-1384, 1975.

Veazie L, Brownlee I and Sear HJ. An outbreak of gastrointeritis associated with Giardia lamblia. In: Waterborne transmission of giardiasis. USEPA, Cincinnati, EPA 600/19-79-001, 1979.

Webster BH. Human infection with Giardia lamblia: analysis of 32 cases. Amer Jour Digest Disease 3:64-71, 1958.

Wolfe MS. Managing the patient with giardiasis: clinical diagnostic and therapeutic aspects. In: Waterborne transmission of giardiasis. USEPA, Cincinnati, EPA 600/19-79-001, 1979.

APPENDIX

Electron Microscopy of *Giardia lamblia* Cysts

DANIEL L. LUCHTEL,* WILLIAM P. LAWRENCE, AND FOPPE B. DEWALLE

Department of Environmental Health, School of Public Health and Community Medicine, University of Washington, Seattle, Washington 98195

The flagellated protozoan *Giardia lamblia* is a recognized public health problem. Intestinal infection can result in acute or chronic diarrhea with associated symptoms in humans. As part of a study to evaluate removal of *G. lamblia* cysts from drinking water by the processes of coagulation and dual-media filtration, we developed a methodology by using 5.0-μm-porosity membrane filters to evaluate the filtration efficiency. We found that recovery rates of *G. lamblia* cysts by membrane filtration varied depending upon the type and diameter of the membrane filter. Examination of membrane-filtered samples by scanning electron microscopy revealed flexible and flattened *G. lamblia* cysts on the filter surface. This feature may be responsible for the low recovery rates with certain filters and, moreover, may have implications in water treatment technology. Formation of the cyst wall is discussed. Electron micrographs of cysts apparently undergoing binary fission and cysts exhibiting a possible bacterial association are shown.

Exposure to the waterborne pathogen *Giardia lamblia* is a current public health problem (10) as exemplified by recent outbreaks of giardiasis reported from Vail, Colo. (7), Berlin, N.H. (15), and Camas, Wash. (11). These outbreaks occurred in municipalities that use surface water for drinking purposes. Each of their seemingly adequate water treatment facilities failed to follow proper treatment procedures of the raw water. *G. lamblia* cysts were detected in the finished water at both Berlin and Camas. The percentages of stool specimens positive for *G. lamblia* cysts reported by U.S. state laboratories in 1976 were 9.2 in California, 9.6 in Colorado, 10.6 in Minnesota, 9.5 in Maine, and 6.3 in Washington (2).

The work reported here is part of a study that determined the efficiency of a water treatment plant for removing *G. lamblia* cysts. Experiments showed that >99% of the cysts introduced into a water treatment pilot plant can be removed by the processes of coagulation-flocculation, sedimentation, and dual-media filtration (W. P. Lawrence, Masters thesis, University of Washington, Seattle, 1979). The efficiency of cyst removal was evaluated by filtering the finished water from the pilot plant. In also evaluating the reproducibility of our filtration procedure with known concentrations of cysts, we found that the recovery rates of cysts that were passed through two different types (Millipore and Nuclepore) and diameters (47 and 293 mm) of membrane filters varied considerably.

Electron microscopy was used to determine the possible causes of these various rates. We found that the cyst wall of *G. lamblia* is remarkably flexible and concluded that the interaction of the flexible cyst wall in the filter pore may explain the different recovery rates on different types and sizes of filters.

MATERIALS AND METHODS

Fecal material was collected from human giardiasis patients in cooperation with the Washington State Parasitology Laboratory, Seattle. The material was fixed in either 5% buffered Formalin or 2% glutaraldehyde in 0.1 M cacodylate, which was done immediately after positive identification of *G. lamblia* cysts in the feces. A given quantity of the fecal material was diluted 1:2 in distilled water, stirred into a liquid suspension, and filtered through three layers of gauze that approximated a 50- to 80-μm-mesh sieve. The filtrate was centrifuged at 400 × *g*. After the supernatant was decanted, the sediment was emulsified with an equal amount of distilled water.

We used the method of Sheffield and Bjorvatn (20) to further separate the cysts from other fecal material. A 5-ml amount of the fecal suspension was added to a discontinuous density sucrose gradient consisting of 5 ml each of 1.5, 1.0, 0.75, and 0.5 M sucrose solutions added successively to a 40-ml conical centrifuge tube. After centrifugation for 30 min at 1,000 × *g*, approximately 4 ml was collected by capillary pipette from both the water-0.5 M sucrose and 0.5 M–0.75 M sucrose interfaces. This suspension, consisting of cysts and small noncyst particulate debris, was diluted 10-fold with distilled water and centrifuged for 3 to 5 min at 400 × *g*. The sediment, consisting of a high number of cysts relatively free of debris, was again diluted 10-fold with distilled water and kept at 4°C until use. We eliminated the final filtration, as recommended by Sheffield and Bjorvatn (20), through a 20-μm filter to remove any remaining debris.

Known quantities of cysts were added to an experimental water supply and tested in a pilot water treat-

*Reprinted with permission from Applied and Environmental Microbiology, Oct. 1980, Vol. 40, No. 4, pp. 821-832.

ment plant for the efficiency of cyst removal (W. P. Lawrence, Masters thesis, University of Washington, Seattle, 1979). It was necessary to develop a quantitative method with a known recovery efficiency that would retain any cysts still remaining in the finished water after passing through the water treatment plant.

We developed a recovery method that used membrane filters of 5-μm pore size to retain *G. lamblia* cysts. We first tested two filters of a small diameter (47 mm). We soon found that it was necessary to test more expensive, larger-diameter filters (293 mm) to maintain filtering efficiency for the relatively large volumes of water from the treatment plant. The recovery efficiency of the filters was tested in the following way.

Aqueous suspensions of fixed *G. lamblia* cysts were passed by vacuum through 5.0-μm-porosity Millipore (Millipore Corp., Bedford, Mass.) or 5.0-μm-porosity Nuclepore (Nuclepore Corp., Pleasanton, Calif.) membrane filters. Concentrations of cysts before and after filtration were determined by enumeration on a Clay-Adams model 4011 Spencer Bright Line hemacytometer and collaborated with counts on a Coulter Counter (Coulter Electronics, Hialeah, Fla.). Cysts were removed from the 47-mm filters by immersing each filter in 10 ml of distilled water in a small flask and agitating gently by hand. The filter was then discarded, and the liquid was examined for presence and quantity of *G. lamblia* cysts. The larger 293-mm membrane filters were processed by using a two-step centrifugation process summarized in Fig. 1. Recovery rates of cysts from the different types and diameters of filters were then calculated.

For the scanning electron microscopic studies, aqueous suspensions of fixed *G. lamblia* cysts were filtered by gravity through 47-mm-diameter 5.0-μm-porosity Millipore or Nuclepore membrane filters. The filters were air dried, and small pieces of the filters were cut out and stuck onto stubs covered with double-stick tape. Other cyst suspensions were critical point dried to avoid membrane filtration and air drying. The aqueous suspensions were postfixed in 1% OsO_4 in 0.15 M cacodylate, dehydrated in ethanol, and critical-point dried with CO_2. After each step of the postfixation and dehydration procedure, the suspensions were briefly centrifuged, and the fluid was decanted. For the critical-point drying step, the suspensions were enclosed in BEEM capsules (Better Equipment for Electron Microscopy, Inc., Bronx, N.Y.) capped on the two ends with 5.0-μm-porosity Nuclepore filters (a modification of the procedure of Hayunga [8]). After critical-point drying, the BEEM capsules were opened, and the dried cysts were sprinkled onto stubs covered with double-stick tape. The stubs were coated with gold-palladium in a Denton Vacuum Desk-1 sputter coater and viewed in a JEOL JSM-35 scanning electron microscope (JEOL, Tokyo, Japan).

20 liters of prefiltered (5.0 μm) tap water
↓
Pass through 293-mm (5.0-μm-pore size)
Nuclepore filter at 10 lb/in^2 with nitrogen
gas. 0.2-μm filter on nitrogen tank
↓
Filter removed and placed in shallow dish.
Cysts washed off by agitation of filter in 0.3
liter of water (platform shaker,
Toothmaster Co., Racine, Wis.)
↓
Centrifuge retentate at 1,500 rpm for 10
min in eight 50-ml conical bottom tubes
↓
Retain "sediment" (approximately
10 ml × 8)
↓
Transfer to two 50-ml conical tubes and
recentrifuge
↓
Retain "sediment" (approximately
5 ml × 2)
↓
Enumerate on Coulter Counter and
compare with initial concentration

FIG. 1. *Summary of method used for the recovery of G. lamblia cysts from 293-mm-diameter 5.0-μm-porosity Nuclepore filters.*

For the transmission electron microscopy studies, aqueous suspensions of fixed *G. lamblia* cysts were postfixed in osmium, dehydrated in ethanol, and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate and viewed with a JEOL JEM 100S electron microscope.

RESULTS

Since a subsequent part of the overall study is concerned with the efficiency of a water treatment pilot plant for the removal of *G. lamblia* cysts (Lawrence and DeWalle, manuscript in preparation), we needed to develop and evaluate a quantitative method with a known recovery efficiency that could be used to determine the number of cysts in a given volume of water. Known quantities of cysts were filtered, and the recovery efficiency was determined. Four different methods were checked against each other.

Recovery rates of *G. lamblia* cysts with the 47-mm-diameter 5.0-μm-porosity Millipore and Nuclepore filters were comparable (Fig. 2). The same recovery rate, approximately 75%, was found when the 293-mm-diameter Nuclepore filter was used (Fig. 3). A significantly lower recovery rate, approximately 25%, was found after filtering cysts with the 293-mm-diameter Millipore filter. Coulter Counter and hemacytometer counts of the filtrates showed that no cysts passed through the filters. The reasons for the less than 100% recovery from the filters and the strikingly lower recovery on the large Millipore filter were unclear. Therefore, it was decided to study the filter surface with scanning electron microscopy.

Cysts collected on either air-dried Millipore or Nuclepore membrane filters exhibited distorted or flattened cyst walls (Fig. 4 to 11). The pattern of such flattening of the cyst wall was

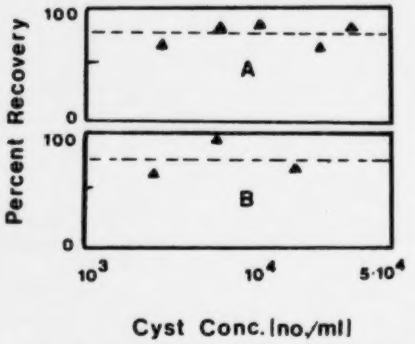


FIG. 2. *Recovery rates (▲) of G. lamblia cysts from (A) 47-mm Nuclepore 5.0-µm-porosity filters and (B) 47-mm Millipore 5.0-µm-porosity filters.*

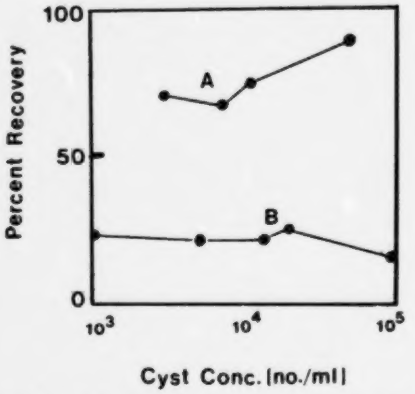


FIG. 3. *Recovery rates (●) of G. lamblia cysts from (A) 293-mm Nuclepore 5.0-µm-porosity filters and (B) 293-mm Millipore 5.0-µm-porosity filters.*

different for cysts collected on Millipore filters compared with cysts on Nuclepore filters. The surface of the Millipore filter consists of intermeshed strands (Fig. 4 and 5), and the diameter of the individual strands is much smaller than the 5.0-µm pore size. The distortion of the cyst on the Millipore surface seemed to be determined to some extent by how it rested on the small individual strands (Fig. 5). For the cysts retained on the surface of a Nuclepore filter, the pattern of distortion was distinctly different (Fig. 6 and 7), apparently because of the smoothness of the Nuclepore surface. A fairly uniform, rimlike structure was apparent around those cysts that rested on the flat surface of the filter (Fig. 7 and 8). Cysts that overlapped the filter pore were sharply bent into the pores (Fig. 6, 10, and 11). Overall, more cysts per unit of area were readily seen on the Nuclepore than on the Millipore filters. Although there seemed to be fewer cysts on the Millipore filters, it was more difficult to detect the cysts on the rough Millipore surface.

We observed sectioned material by transmission electron microscopy (Fig. 12) to confirm the presence of *Giardia* cysts. Cysts prepared via critical-point drying were not flattened (Fig. 13; see also Fig. 14 to 17). Rather, such specimens appeared ovoid or spherical and agreed with the transmission electron microscopic observations. The possible forces that may act on cysts to distort them during the processes of filtration and air drying are considered below.

Some additional observations were made on the material that had been prepared for electron microscopy. Some of the cysts appeared to show a process of division (Fig. 8 and 9). One "stretched" cyst was found, apparently an artifact caused by the preparative procedures (Fig. 10).

With the scanning electron microscope, a variety of material was observed on the cyst wall. This was particularly evident on critical-point-dried specimens (Fig. 13 to 17). Air-dried cysts were usually free of such material (Fig. 6). Occasionally, bacterium-like structures were associated with the cysts (at the upper right and lower left of the double cyst shown in Fig 10 and at the right of the cyst shown in Fig. 15). One cyst in the sections prepared for transmission electron microscopy showed a bacterium-like structure associated with the cyst wall (Fig. 18).

Our transmission electron microscopic preparations usually showed a rather wide space between the organism and the cyst wall (Fig. 12 and 19). A peripheral array of vesicles was characteristic for most organisms. A dense-staining material coated the inside surface of these peripheral vesicles. A few larger peripheral lacunae were seen (asterisk in Fig. 19). The inner surfaces of the lacunae were lined with a dense-staining material. A dense material also coated the inner surface of the cyst wall and the surface of the encysted organism.

DISCUSSION

Information about the biology of *Giardia* organisms, the incidence of giardiasis, and the ultrastructure of these parasitic protozoans is reviewed in three recent publications (1, 10, 13). Several scanning electron microscopy studies on the trophozoite (4, 17, 23) complement transmission electron microscopy studies (3, 6, 18, 19; additional references in 13). Previous ultrastructural studies of the cyst are those of Sheffield and Bjorvatn (20), Sheffield (19), and Tombes et al. (21).

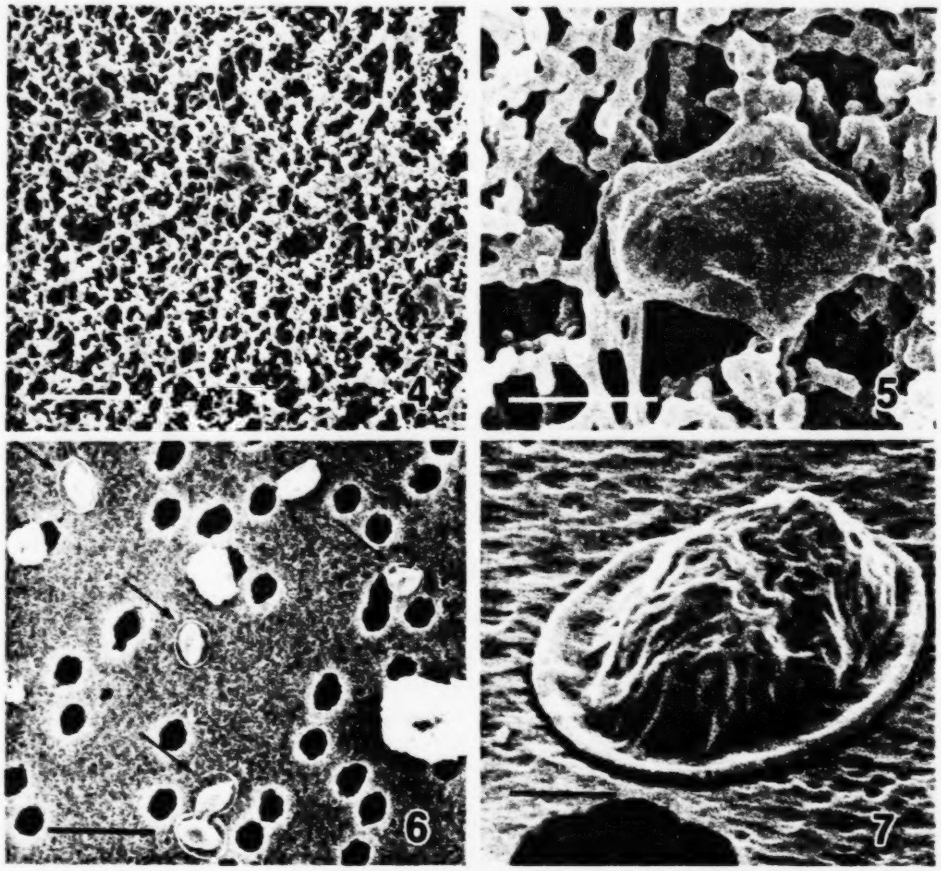


FIG. 4–7. *Scanning electron micrographs showing cysts collected on filters by gravity filtration and then air dried.*

FIG. 4. *A low-magnification view that shows three G. lamblia cysts (arrows) on a 5.0-µm-porosity Millipore filter. Bar, 20 µm.*

FIG. 5. *A higher-magnification view of the middle cyst shown in Fig. 4. The cyst is flattened and distorted. The distortions seem to depend on how the cyst rests on the contours of the filter surface. Bar, 5 µm.*

FIG. 6. *A low-magnification view of a 5.0-µm-porosity Nuclepore filter that shows several cysts (arrows) and some unidentified debris, presumably consisting of fecal material and ruptured cysts (arrowhead). Bar, 20 µm.*

FIG. 7. *A higher-magnification view of a cyst, comparable to those shown in Fig. 6. The cyst is flattened on the filter surface and typically shows a thin outer rim or flange. The central convex portion of the cyst is caused by the encysted organism. Bar, 2 µm.*

Flexible cyst wall. Although the above ultrastructural studies (and this study) provide detailed information about the structure of the trophozoite and the cyst, it seems worthwhile to begin this discussion by referring to the earlier work of Filice (5), who observed fresh, unfixed preparations of cysts. He noted that the cyst is a flexible structure since he saw that the organism could move about inside and deform the cyst wall. He also observed that the cyst wall had enough strength to keep its shape when the protoplasm within disintegrates and, also, that the cysts do not explode when immersed in distilled water.

The most striking feature of the cyst wall shown in our initial observations on air-dried preparations is its flexible nature, even after the cyst is fixed in glutaraldehyde or Formalin. Such

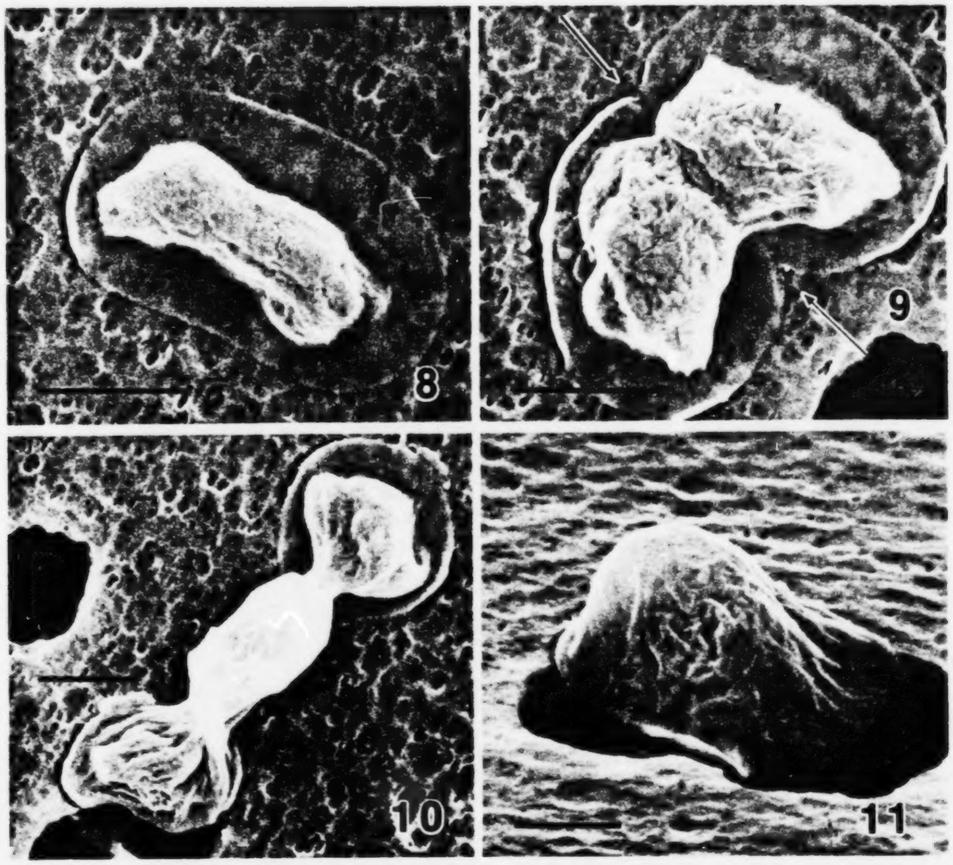


Fig. 8–11. *Air-dried cysts collected on Nuclepore filters. Apparently, encysted organisms are able to divide, and the cyst wall is then restructured to enclose separately each of the two newly formed organisms.*

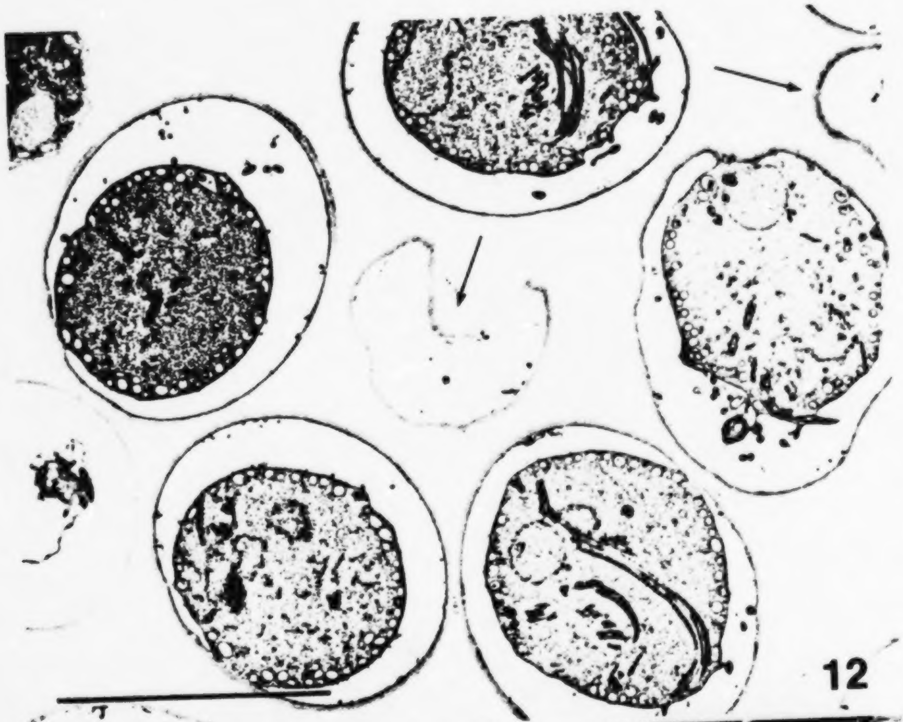
Fig. 8. *A single cyst in which the organism inside appears to be in the process of dividing. Bar, 5 µm.*

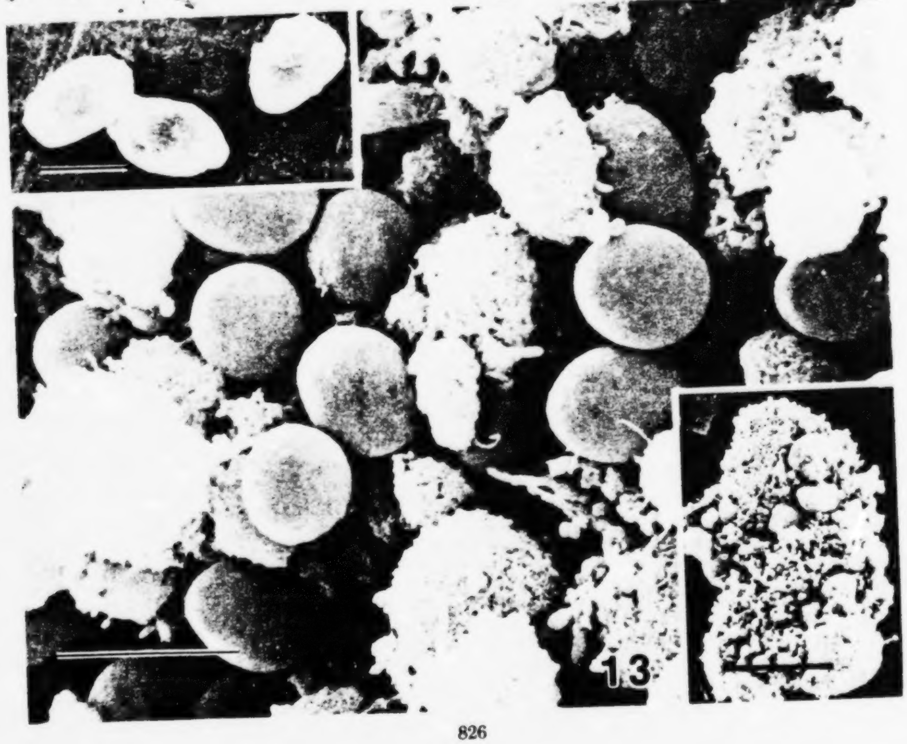
Fig. 9. *A double cyst, apparently formed after an organism within a single cyst had divided. The arrows indicate a line of demarcation that separates the two cysts. Presumably, this double cyst breaks apart to form two separate cysts. Bar, 5 µm.*

Fig. 10. *A double cyst that has been stretched artifactually during the preparation and filtration procedures. Bar, 5 µm.*

Fig. 11. *A cyst that has become distorted, apparently because of settling into a pore of the filter. Bar, 2 µm.*

flattened shapes for *Giardia* cysts (Fig. 5 and 7) are not consistent with the ovoid outlines of cysts shown by various light microscopic studies (13) and the transmission electron microscopy observations of Sheffield and Bjorvatn (20). We then confirmed that our material was *Giardia* cysts by transmission electron microscopy (Fig. 12) and subsequently showed that ovoid cysts could be prepared for scanning electron microscopic observation if the cysts are critical-point dried (Fig. 13). Although we did not check each set of variables independently (filtering versus not filtering; air drying versus critical-point drying), most of the flattening is probably due to the surface tension of water as the specimen is being air dried. Some of the critical-point-dried cysts were somewhat distorted (insert, Fig. 13), possibly due to some transient air drying during the several fluid exchanges before the critical-point-drying step. But overall, although the critical-point-dried cysts underwent several filtering and centrifugation steps, they retained their ovoid shape. On the other hand, filtration had some effect on the cyst morphology as the


12


13

cysts appeared distinctly different on the Millipore surface (Fig. 5) compared with those on the Nuclepore surface (Fig. 7).

Tombes et al. (21) studied the cysts of *Giardia* collected from a variety of mammals, including humans. The morphology of the cysts they collected from humans is different from that observed by us. The cysts they studied by phase microscopy had the typical elliptical shape; by scanning electron microscopy, the cysts seemed to be distorted, having a cuboidal shape. Possible reasons for our different results are difficult to decide upon since Tombes et al. used a variety of fixation and preparative techniques, and for any particular micrograph, the data are not given as to how the cysts were fixed, whether the material was fixed immediately or after some initial filtrations (sucrose flotation techniques were not used), how long the material was stored in aldehyde before drying, and whether the cysts were air dried or critical-point dried. Overall, Tombes et al. noted no consistent differences in cysts after air or critical-point drying. We found substantial differences in cyst morphology when cysts were air dried or critical-point dried. We suggest that a possible procedural error that Tombes et al. mention in their discussion may be a significant factor in our different results.

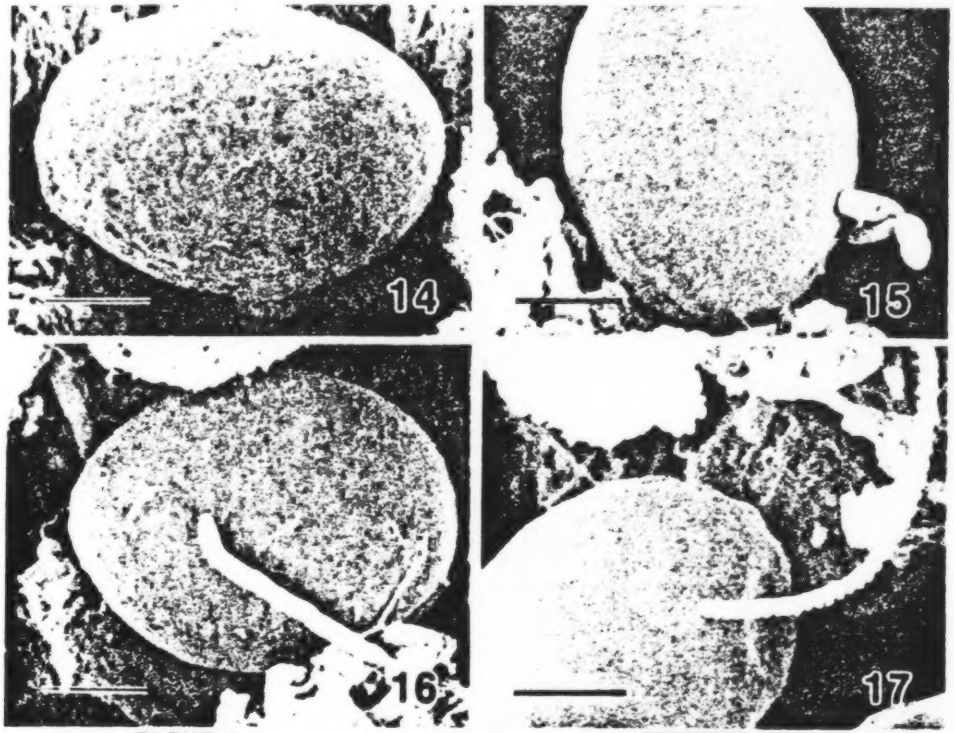
Sucrose flotation technique. We used the sucrose flotation method of Sheffield and Bjorvatn (20) to prepare suspensions of cysts. They apparently fixed the cysts after the sucrose procedure. If so, they obtained remarkably good fixation after a lengthy concentration process. We fixed the fecal material before the sucrose flotation. For laboratory diagnosis of giardiasis in unfixed stools, the basic method is a zinc sulfate flotation method (13). With this technique, the cytoplasm of the cells is plasmolyzed by the hypertonic zinc sulfate solution, and the cytoplasm is characteristically concentrated at one side of the cyst (see Fig. 23 in reference 13). Although the cyst wall is apparently stable throughout the zinc sulfate flotation process, it seems much more delicate when sucrose flotation is used. Levine (14) observed that *Giardia* cysts concentrated by sugar flotation shrivel and become unrecognizable in a matter of minutes. Stevens, in a discussion after Levine's paper (14), noted that there was no morphological effect on the cysts with the sucrose flotation technique if the cysts were removed immediately from the interface and placed in physiological saline. With the methodology of Sheffield and Bjorvatn (20), the suspensions are diluted 10-fold with water after collecting them from the interfaces.

Another possible effect of the sucrose flotation method is that it may change the width of the space between the cyst wall and the organism. Sheffield (19) believes that these spaces are not caused by the different isotonic pressures of the flotation solutions. We found a much wider space between the cyst wall and the organism than that shown by Sheffield and co-workers (19, 20) or the cyst shown by transmission electron microscopy in the study of Nemanic et al. (18). The material studied by Nemanic et al. (18) was not exposed to a sucrose flotation technique as the organisms were prepared for electron microscopy by washing pieces of gut and centrifuging the wash. Perhaps species differences may be a factor in comparing our results with those of Nemanic et al. (18) but the reasons for our results being different from those of Sheffield and Bjorvatn (20) are not apparent unless they fixed the cysts after sucrose flotation. Perhaps selection of micrographs may be a contributing factor as Sheffield, in a discussion after his paper (19), states that a variety of cyst types were seen; that is, cysts in which the cytoplasm was closely applied to the cyst wall, whereas others showed large, open areas between cytoplasm and wall. We also saw sections of cysts in which the cytoplasm was closely applied to the cyst wall, but since most of the sectioned cysts showed an open space (Fig. 12), our interpretation is that the organism does not occupy the entire space of the cyst. The rimlike structure on air-dried cysts (Fig. 7) would also indicate that the cyst wall collapsed into a space not occupied by the encysted organism. We observed that the cyst walls are usually 0.15 to 0.25 μm thick, which is less than the 0.3-μm thickness observed by Sheffield and Bjorvatn (20).

Composition of cyst wall. The composition of the cyst wall is unknown. Filice (5) was not able to obtain any positive histochemical information, although he did show that it was Feul-

FIG. 12. *A transmission electron micrograph of encysted Giardia organisms. The cyst walls usually form smooth ovoid outlines, although a couple of examples of acutely folded cyst walls (arrows) can be seen (also see insert of Fig. 18). Bar, 10 μm.*

FIG. 13. *Smooth, ovoid cysts after critical-point drying. These cysts are embedded in a mat or clump of debris, bacteria, and fecal material. A low-magnification micrograph of the entire clump is shown in the lower right insert. The upper left insert shows examples of single, isolated cysts after critical-point drying. Such cysts may show some moderate degree of distortion. Bar, 10 μm. Lower right insert bar, 100 μm. Upper left insert bar, 5 μm.*



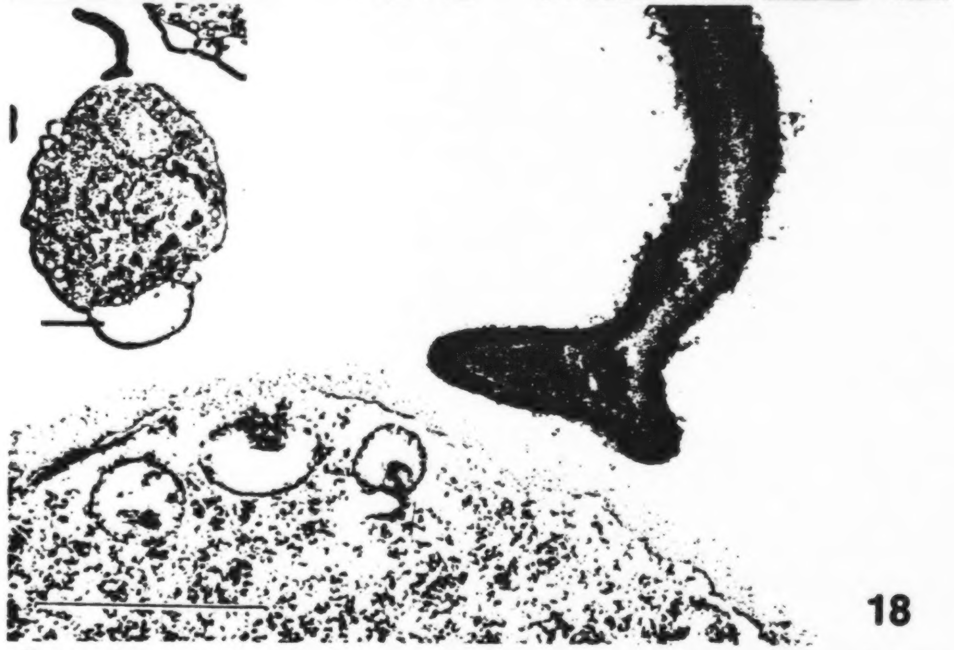


Fig. 14–17. *A variety of cyst morphologies as seen after critical point drying. Almost all cysts had some sort of material or debris stuck on the cyst wall. In some cases, structures that could be identified as bacteria were attached to the cyst wall (Fig. 15). In other cases, unidentified fibrous forms were seen on the cyst walls (Fig. 16 and 17). Bars, 2 µm.*

Fig. 18. *A transmission electron micrograph showing a structure, presumably bacterial in nature, attached to the cyst wall. The fibrous coat of the attached structure seems to interact with the fibrous cyst wall. The insert shows a low magnification view of the entire cyst and attached structure. Bar, 1 µm. Insert bar, 2 µm.*

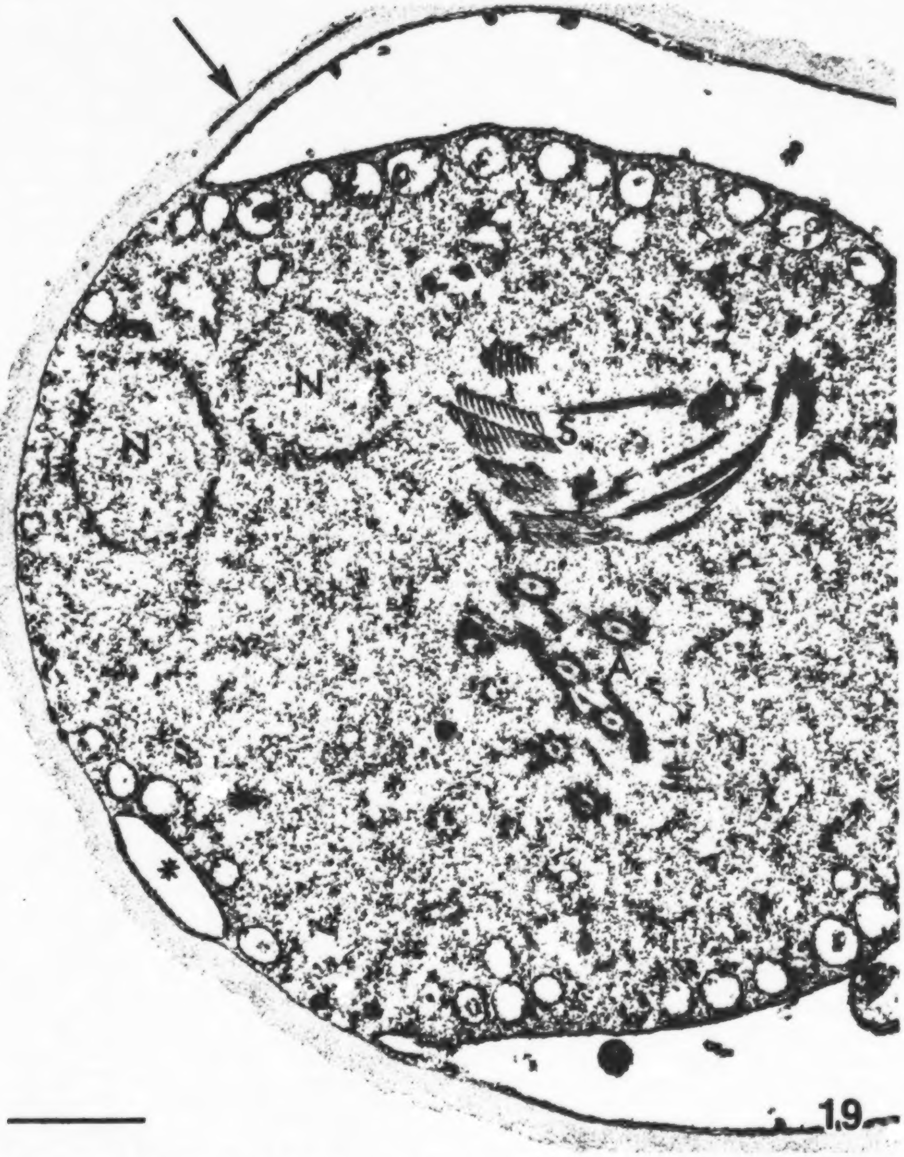


FIG. 19. *An encysted organism with its typical array of peripheral vesicles (also see Fig. 12). N, nuclei; A, axonemes of the flagellae; S, microtubule-ribbon complexes of the fragmented sucking disk. The arrow points to a portion of the cyst wall that has apparently retained a staining density similar to the staining density of the inner surface of the cyst wall. The asterisk is in a peripheral lacuna. Bar, 1 µm.*

gen stain negative; it did not stain with a lipid stain, Sudan IV, and it did not seem to be affected by various enzyme digestions (pepsin, trypsin, and papain). In any case, the cyst wall is not fixed adequately with aldehydes to withstand the surface tension of water during air drying, and its flexible nature, even after fixation, may lower the filtering efficiency of various water filtration plants. The nature of the cyst wall needs to be taken into consideration when various cyst model systems are being tested. For example, Logsdon et al. (16) used 9-μm-diameter radioactive microspheres as a model for *Giardia* cysts because the cysts are difficult to obtain, detect, and count, whereas the radioactive microspheres are similar in size to *Giardia* cysts and are easy to trace. Our observations suggest that such microspheres would be filtered more efficiently than *Giardia* cysts in pilot water filtration plants.

Loss of cysts during membrane filtration. The maximum rate of recovery obtained from the Millipore and Nuclepore membrane filters was 75%. A number of factors may account for the 25% loss. Cysts may remain attached or embedded in the filter after the recovery procedure, adhere to nonfilter surfaces of the filtration assembly, pass through the filter, or be destroyed during the filtration or centrifugation process or both.

The filters were agitated by hand as vigorously as possible without destroying the filters. It was later suggested that perhaps a better method would be to vigorously and systematically wash the filter surfaces with strong streams of distilled water with 0.01% Tween 20 from a capillary pipette. We did not test such a washing procedure. Compared with the unidimensional surface of the Nuclepore filter, the convoluted fibrous structure of the Millipore filter may permit cysts and other material to become embedded within the depth of the filter, and, by our recovery procedure, the cysts would not be readily washed out. Such differences in the filter characteristics may explain the difference in recovery rates between the 293-mm-diameter Millipore filter and the 293-mm-diameter Nuclepore filter. What is still puzzling are the comparable recovery rates of the 47-mm-diameter Millipore and the 47-mm-diameter Nuclepore filters. However, a 293-mm-diameter filter has approximately 39 times more surface area than a 47-mm-diameter filter. Thus, although there may be some difference in recovery rates for the 47-mm-diameter Millipore and Nuclepore filters, perhaps we were not able to detect this difference until the larger surface area of the large-diameter filter (with its larger number of cysts) made it apparent.

Part of the overall 25% loss could be attributable to cysts passing through the filters. The flexibility of the cysts is suggestive evidence for how cysts could pass through individual pores of smaller diameter than the size of the cysts (Fig. 11). With the optical and Coulter Counter methods used, however, no cysts were detected in the filtrates. In our removal study (W. P. Lawrence, Masters thesis, University of Washington, Seattle, 1979), some *G. lamblia* cysts were found to pass through a 4-ft column of dual-media filters (sand and anthracite) before any turbidity breakthrough, indicating that the filter column was still intact. Although an equivalent pore size cannot be determined in dual-media filters, that *G. lamblia* cysts can somehow penetrate the dual-media filter again indicates their flexible nature.

Some cysts are probably lost because they are destroyed during the preparative steps. What is probably a remnant of a cyst is shown in Fig. 6. Overall, destruction of cysts probably accounts for most of the 25% loss of cysts during the recovery procedure on membrane filters. A further loss occurs with the Millipore filter, probably because of the embedding of cysts in the filter.

Formation of cyst wall. The staining density of the material lining the inner surfaces of the peripheral vesicles and lacunae is similar to the staining density of the material on the surface of the encysted organism and the inner surface of the cyst wall (Fig. 18 and 19). The vesicles thus seem to contain a secretory material that eventually is used to make the cyst wall. The cyst wall has a fibrous substructure, as if it were formed by successive layers of material. We suggest that the successive layers arise from successive waves of vesicles that coalesce to form enlarging peripheral lacunae. Eventually, one giant lacuna in effect completely surrounds the organism, and, in the process, another layer of the cyst wall has been laid down. By some type of maturational process, the newly formed layer of the cyst wall then loses much of its staining density. Occasionally, however, the staining density is retained, as indicated by the arrow in Fig. 19.

Hemmes and Hohl (9) ventured a similar hypothesis for encystment of *Phytophthora parasitica* zoospores. Encystment involved the fusion of peripheral vesicles with the plasmalemma, followed by the release of glycoprotein and possibly other cell wall precursor materials. Friend (6) suggested that the location of the peripheral vacuoles in the trophozoites of *Giardia muris* were consistent with a secretory function, perhaps the secretion of the cyst wall. Mucocytes in other protozoa are rows of globular elements beneath the pellicle that discharge gelatinous or mucoid secretions (6). Finally, Filice (5) observed that, in living organisms, the cyst forms first on the dorsal surface of the trophozoite from refractile granules in the peripheral cytoplasm. On the other hand, Sheffield (19) discounted the role of the peripheral vesicles as secretory vesicles involved in cyst wall formation since he noted their abundance after wall formation. Although our observations also showed this abundance of vesicles (Fig. 12 and 19), their

J. C. Hoff (ed.), Waterborne transmission of giardiasis. U.S. Environmental Protection Agency, Cincinnati, Ohio.

15. **Lippy, E. C.** 1978. Tracing a giardiasis outbreak at Berlin, New Hampshire. J. Am. Water Works Assoc. **70:**512–520.

16. **Logsdon, G. S., J. M. Symons, and R. L. Hoye.** 1979. Water filtration techniques for removal of cysts and cyst models, p. 240–256. *In* W. Jakubowski and J. C. Hoff (ed.), Waterborne transmission of giardiasis. U.S. Environmental Protection Agency, Cincinnati, Ohio.

17. **Mueller, J. C.** 1973. Scanning electron microscope observations in human giardiasis. Scanning Electron Microsc. **1973:**557–564.

18. **Nemanic, P. C., R. L. Owen, D. P. Stevens, and J. C. Mueller.** 1979. Ultrastructural observations on giardiasis in a mouse model. II. Endosymbiosis and organelle distribution in *Giardia muris* and *Giardia lamblia*. J. Infect. Dis. **140:**222–228.

19. **Sheffield, H. G.** 1979. The ultrastructural aspects of *Giardia*, p. 9–21. *In* W. Jakubowski and J. C. Hoff (ed.), Waterborne transmission of giardiasis. U.S. Environmental Protection Agency, Cincinnati, Ohio.

20. **Sheffield, H. G., and B. Bjorvatn.** 1977. Ultrastructure of the cyst of *Giardia lamblia*. Am. J. Trop. Med. Hyg. **26:**23–30.

21. **Tombes, A. S., S. S. Landfried, and L. D. Williams.** 1979. Surface morphology of *Giardia* cysts recovered from a variety of hosts, p. 22–37. *In* W. Jakubowski and J. C. Hoff (ed.), Waterborne transmission of giardiasis. U.S. Environmental Protection Agency, Cincinnati, Ohio.

22. **Trager, W.** 1964. The cytoplasm of protozoa, p. 81–137. *In* J. Brachet and A. E. Mirsky (ed.), The cell, vol. 6. Academic Press, Inc., New York.

23. **Watson, J. H. L., J. Goodwin, and K. S. Rajan.** 1979. *Giardia lamblia* in human duodenum and bile. Micron **10:**61–64.